

37th EASD Annual Meeting of the European Association for the Study of Diabetes

Glasgow, United Kingdom, 9–13 September 2001

Abstracts

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OP 1

Diabetic Foot: Aetiology and Pathogenesis

1

MOBILITY OF THE FOOT-ANKLE COMPLEX IN DIABETIC NEUROPATHIC PATIENTS

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Background and Aims: Peripheral neuropathy induces significant alterations in the foot-floor interaction during gait; furthermore, with the increase of neuropathy a 'hip' ankle strategy replaces an 'ankle' strategy. The last phenomenon could be associated with limited mobility of the ankle articular complex. The aim of the study is to accurately measure the angular excursions of the ankle articular complex in the sagittal, transversal and frontal planes.

Materials and Methods: We evaluated 65 patients: 21 diabetics without neuropathy (D), 15 with neuropathy (DN), 16 with previous neuropathic ulcer (DPU) and 13 healthy subjects (C). For each patient but for DPU patients, the contributions of the two legs have been considered as two different cases. For DPU patients, instead, only the leg corresponding to the previous ulcerated foot has been included in the study. A dedicated instrument (angular resolution 0.05° in the sagittal plane; 0.02° in the transversal and frontal planes) has been used for the angular measurements, whose peculiarity is to allow the performance of the rotations around each of the three anatomical axes separately. The origin of the reference system lies in the intersection between the tibial axis (vertical axis) and the medio-lateral axis passing through the malleoli.

Results: A progressive remarkable reduction of dorsal and plantar flexion has been found in D, DN and DPU patients (dorsal flexion: 33.42±8.4° for C, 27.80±6.7° for D, 29.37±6.7° for DN, 26.16±4.9° for DPU; plantar flexion: 38.40±8.2° for C, 35.00±8.2° for D, 31.34±7.1° for DN, 32.35±4.4° for DPU). Significant reductions of the excursions in the other two planes have been found only for DPU patients (excursion in the transversal plane: 75.98±19.7° for C, 65.94±22.0° for D, 67.83±16.4° for DN, 58.98±17.5° for DPU; excursion in the frontal plane: 44.87±10.7° for C, 44.15±13.0° for D, 45.21±10.5° for DN, 35.77±5.7° for DPU).

Conclusions: The reduction of the articular mobility of the foot-ankle complex, especially in the sagittal plane, confirms the hypothesis of a change in the locomotor strategy, which implies a major involvement of the hip joint.

3

Matrix metalloproteinase and tissue inhibitor of metalloproteinase expression in diabetic and venous ulcers

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Background and Aims: Matrix metalloproteinases (MMP) cause excessive tissue breakdown and are regulated in vivo by tissue inhibitor of metalloproteinases (TIMP), their natural inhibitors. In venous leg ulcer (VLU), an imbalance between proteolytic enzymes and proteinase inhibitors, may be a factor in delayed healing. Little is known of the pattern of expression in diabetic foot ulcers (DFU) of MMPs and TIMPs.

Patients and Methods: Biopsies were taken from the dorsum of the foot of non-diabetic and diabetic neuropathic subjects and from the edge of VLU and neuropathic DFU. Using monoclonal antibodies, we stained frozen tissue sections to investigate the expression of MMPs-1, -2, -8, -9, TIMPs-1 and -2 in normal and diabetic skin (NS and DS, respectively), DFU and VLU.

Results: There was excessive staining for all MMPs and TIMPs in the base of all ulcers examined, with MMP-8 and TIMP-2 showing intense staining. MMP-9 expression was particularly strong within VLU. MMPs-1 and -8 were expressed in NS and DS, particularly in epidermal cells. Increased MMP-8 staining was seen in the dermis adjacent to DFU and VLU. There also appeared to be increased basal epidermal cell staining for MMP-1 and TIMP-2 at the ulcer margins. No TIMP-1 staining was seen in NS or DS, however, TIMP-2 was located weakly in the dermis and along regions of the epidermal-dermal basement membrane. Within the ulcer base of DFU and VLU, MMP and TIMP expression was similar. However, at the edge of VLU there was enhanced staining of MMPs-2, -8, -9 and TIMP-1 in the dermis under the epidermal leading edge and MMP-2 appeared markedly expressed in suprabasal layers of the adjacent epidermis.

Conclusions: MMP and TIMP expression appears elevated in chronic wounds and surrounding skin. Excessive expression of proteinases and their inhibitors may play a role in the non-healing nature of chronic wounds by upsetting the proteinase-inhibitor balance necessary for healing to occur.

2

THE EFFECTS OF AMINOGLUCANIDINE AND BETA-1,3-D POLYGLUCOSE ON WOUND HEALING IN DIABETES MELLITUS.

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Background and Aims: Healing of diabetic wounds is delayed, partly by way of altered cellular mechanisms in the healing wound, and also possibly because of alterations in extracellular matrix proteins due to non-enzymatic glycosylation. Macrophages infiltrate the wound and take part in the inflammatory response by producing cytokines like Tumor Necrosis Factor- α (TNF- α), and they also internalize advanced glycation end-product (AGE)-modified proteins. The present study was undertaken to compare the effects of the glycation inhibitor, aminoguanidine (AG), in the early onset of diabetes, and the macrophage stimulating agent, beta-1,3-D polyglucose (BDP), on wound healing, compared to insulin treatment alone and placebo controls.

Materials and Methods: Cutaneous wounds were established on the back of either diabetic (C57BL/6J-db/db) or non-diabetic (C57BL/6J+/+) mice. Five groups were studied: 1 db/db-mice with placebo wound treatment (n=23, average blood glucose (BG) 32.5 ± 1.4 mmol/l; (SEM). Average HbA_{1c} 12.1 ± 0.4% (n=15); (SEM). 2 db/db-mice treated with insulin s.c. (n=12, BG 18.6±3.2 mmol/l, HbA_{1c} 7.7±0.2 %). 3 db/db-mice given AG 0.1% in the drinking water (n=12, BG 31.7 ± 1.9 mmol/l, HbA_{1c} 13.7±0.3 %). 4 db/db-mice treated with a combination of insulin s.c. and multiple doses of BDP (n=11, BG 14.3±2.5 mmol/l, HbA_{1c} 8.0±0.5 %). 5 non-diabetic control mice without treatment (n=15, average BG 8.3±0.5 mmol/l, average HbA_{1c} 3.9±0.04% (n=7)).

Results: The LPS-stimulated release of TNF- α from isolated peritoneal macrophages was only 1/8 of that from non-diabetic mice. The percentage reduction in wound area after 10 days for group 1-5 was: 3±2%, 1±1%, 4±1%, 10±2%, and 69±3 % (mean±SEM, *p<0.05 vs. normal controls, †p<0.05 vs. diabetic controls). The corresponding results after 13 days were: 13±3%, 11±4%, 15±1%, 32±7%, and 87±2 %.

Conclusions: Macrophage function, as judged by TNF- α release, is impaired in diabetes. Neither improved metabolic control nor oral intake of aminoguanidine accelerate wound healing in this model. However, topical applications of BDP improve wound healing significantly in diabetic mice, despite moderate hyperglycemia.

4

PLASMA LEVELS AND PLATELET EXPRESSION OF P-SELECTIN IN TYPE 2 DIABETICS WITH FOOT ULCERS

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Background and Aims : Previous studies have shown that plasma cell adhesion molecules including P-selectin are increased in patients with diabetes mellitus. The role of these adhesion molecules in platelet activation producing a prothrombotic state and contributing to diabetes-related complications was not extensively studied. **Subjects and Methods :** We investigated the activated platelets with surface P-selectin expression in 20 type 2 diabetics with foot ulcers, 20 type 2 diabetics without ulcers and 20 age and sex-matched healthy control subjects using flow cytometric analysis of platelet rich plasma. In addition plasma P-selectin was estimated using standard immunoassay kits. **Results :** Platelet surface P-selectin expression was higher in type 2 diabetics with foot ulcers (47.9 ± 13.78 positive cells) compared to diabetics without foot ulcers and the control (17.6 ± 15% and 7.02 ± 3.61% positive cells respectively, P<0.01). A parallel increase in plasma P-selectin was found in diabetics with foot ulcer compared to diabetics without foot ulcers and the control (174.28 ± 28.40 ng/ml vs 129.3 ± 16.08 ng/ml and 76.7 ± 11.82 ng/ml, respectively, P<0.05). Plasma level and platelet expression of P-selectin were significantly correlated. Similarly plasma P-selectin correlated positively with the duration of diabetes, total cholesterol, triglycerides, 24 h urinary albumin excretion (24 h UAE) and peroneal nerve conduction velocity (PNCV), but no significant correlation was found between plasma P-selectin and each of glycated haemoglobin (HbA_{1c}) and body mass index (BMI). **Conclusion :** We conclude that plasma P-selectin correlates positively with platelet surface P-selectin expression in diabetics giving a reliable and non-invasive marker of platelet activation. Both plasma level and platelet P-selectin expression are markedly elevated in diabetics with foot ulcers giving a rationale for the early use of antiplatelet drugs in the treatment of this group of patients.

EXPRESSION OF GELATINASE (MMP-2) IN DIABETIC AND NON-DIABETIC WOUNDS
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Background and Aims: Incomplete wound healing and the change from an acute to a chronic wound is the focus of attention with wound healing problems in diabetic patients. Previous studies demonstrated high levels of pro-inflammatory cytokines, high levels of active proteases, especially matrix metalloproteinases (MMPs), and low levels of growth factor activity in chronic pressure ulcers. The data also show that as a chronic wound begins to shift towards a healing wound, the pattern of cytokine, protease, and growth factor activity shifts back to the levels observed in an acute healing wound. **Materials and Methods:** In our study we investigated the expression of MMP 2 pro and active. We compared the data of 24 diabetic patients with diabetic foot lesions (DFS), non-diabetic patients with chronic pressure ulcer (n=3; UC) and in wound tissue of non-diabetic patients with traumatic lesions (n=20; TR). A five mm punch biopsies were taken from the centre of the lesions. After homogenisation (2mlPBS + 1% TritonX-100) we analysed the activity of MMP 2 by zymography (Novex[®]). **Results:** Compared with the healthy patients (after injury) MMP-2_{pro} (p=0.041; DFS 157.8 pg/ml ± 218; TR 54.2 pg/ml ± 75) and MMP-2_{active} (p=0.033; DFS 102.6 pg/ml ± 180; TR 17.1 pg/ml ± 8) was significantly increased in diabetic patients. Patients with chronic pressure ulcer showed the highest levels of MMP compared with diabetic (MMP-2_{pro}: p=0.007; DFS 157.8 pg/ml ± 218; UC 733.4 pg/ml ± 850; MMP-2_{active}: p=0.033; DFS 102.6 pg/ml ± 180; UC 394.3 pg/ml ± 414) or post traumatic healthy patients (MMP-2_{pro}: p=0.002; TR 54.2 pg/ml ± 75; UC 733.4 pg/ml ± 850; MMP-2_{active}: p=0.001; TR 17.1 pg/ml ± 8; UC 394.3 pg/ml ± 414). **Conclusion:** We found a clear difference for MMP 2 (pro and active) expression between chronic (ulcus cruris and diabetic foot ulcers) and non-diabetic traumatic lesions. Our data agree with previous studies of chronic pressure ulcers, in that diabetic foot injuries often fail to heal because of persistently high levels of MMPs. Further studies are needed to investigate the therapeutic effects of growth factors or proteinase inhibitors to prevent the chronification of diabetic foot ulcers.

THE END-STAGE DIABETIC AMPUTATED LEG: A SYNDROME OF TIBIAL ATHEROSCLEROSIS, MEDIAL CALCIFICATION AND AXONAL NEUROPATHY WITH RELATIVE SPARING OF THE FOOT ARTERIES

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Background and Aims: Amputations in diabetic patients continue to be a major source of morbidity. The aim of this study was to quantitate plaque formation, medial calcification and thrombosis in tibial and foot arteries and axonal fibre loss in sural / tibial nerves in amputated legs.

Materials and Methods: The amputated legs of 55 diabetic patients (65±10 years - Mean age ± SD) and 40 non-diabetic control patients (70±14 years) were studied. Blocks were dissected out containing the tibial arteries (posterior tibial and anterior tibial arteries) and foot arteries (dorsalis pedis, medial and lateral plantar arteries) and sural and tibial nerves. Plaque formation, medial calcification, thrombosis and axonal fibre loss was scored from 0-3.

Results: Medial calcification was significantly increased in the diabetic tibial arteries at 1.81±1.13 compared with 0.41±0.80 in controls (p<0.001). Plaque formation was similar in diabetic tibial arteries compared with the controls, 2.40±1.18 versus 2.14±0.88 as was thrombosis 0.81±0.87 versus 1.19±1.14. Mean axonal fibre loss was 2.87±0.23 in diabetic patients compared with 0.29±0.55 in controls (p<0.001). Finally plaque formation was significantly increased in the tibial arteries compared with the foot arteries both in diabetic patients, 2.40±1.18 versus 0.81±1.02 (p<0.001) and in the controls 2.14±0.88 versus 0.69±0.84 (p<0.001).

Conclusions: The diabetic amputated limb is characterised by atherosclerotic plaque and medial calcification in the tibial arteries closely associated with axonal neuropathy which could be important in its pathogenesis. The foot arteries of both diabetic and control patients were relatively spared and maybe more suitable than the tibial arteries for distal bypass.

OP 2

Oral and Pulmonary Insulin Delivery

EARLY INTRODUCTION OF ORAL INSULIN IN PATIENTS FAILING ON DIET AND EXERCISE, A LONG-TERM EFFICACY STUDY

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Background and Aims: The UKPDS Study proved that in type-2 DM glycemic control can decrease tripathy. However, it also showed how sulfonylureas (SU) and Metformin (Met) fail over time. Insulin is always an option for glycemic control in clinic. Needle use is often the limiting factor. The early introduction of pain free oral insulin treatment via the RapidMist may improve glycemic control. This system is based on the unique liquid aerosol formulation, which allows a precise insulin dose delivery in the form of fine aerosolized droplets directed in the mouth. The goal of the study was to evaluate the (90 days) efficacy of the low dose of oral insulin (7 units, absorbable) in patients failing on diet/exercise to improve post-prandial glucose profile and HbA1c. **Materials and Methods:** In a double blind, placebo controlled, randomized study, 33 type-2 diabetic patients failing on diet/exercise received oral insulin (7 units) or placebo puffs via the RapidMist device. The HbA1c levels were measured every month along with the daily glucose profile every week. **Results:** The table below shows HbA1c profiled at selected times

Time (days)	0 days %HbA1c	30 days %HbA1c	60 days %HbA1c	90 days %HbA1c
Diet/Exercise + Placebo Puffs	8.5	8.4	8.7	9.0
Diet/Exercise + Oral Insulin	9.7	8.7	8.1	8.0

Conclusions: We conclude that the HbA1c levels significantly improved (1.7%, p<0.0001) in the group receiving low doses of oral insulin when compared to the controlled group, where HbA1c levels worsened (up by 0.5%, p<0.1638). This preliminary result showed that Oralin could be used in diet and exercise failure patients to achieve better control of glucose levels. The trials are in progress to confirm the long-term efficacy and improved control.

INTENSIVE THERAPY AND PATIENT SATISFACTION IN TYPE 1 DIABETES: A RANDOMIZED TRIAL OF INJECTED VS. INHALED INSULIN

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Background and Aims: Despite strong evidence demonstrating its health benefits, aggressive insulin therapy requiring multiple daily injections (MDI) has been slow to gain acceptance. To quantify the burden of MDI in terms of patient satisfaction and quality of life we studied 327 patients with type 1 diabetes randomized to twice-daily NPH insulin plus either pre-meal Regular insulin injections, n = 165 (MDI) or pre-meal inhaled insulin, n = 162 (INH). **Materials and Methods:** Patients were 53% male, aged 12-65 yrs (mean age 29.5±14.6 yrs), and had mean HbA_{1c}=8.0±1.0%. Quality-of-life and satisfaction questionnaires were completed at baseline and 6, 12, 20 and 24 weeks after treatment. **Results:** The Overall Satisfaction score (scaled 0 to 100) improved substantially more for INH from baseline to endpoint (62.1 to 74.5) than for MDI (62.8 to 64.3), p<0.0001. Changes from baseline to endpoint for all satisfaction subscales (0 to 100) showed similar improvements for INH as compared to MDI. INH change±SE vs MDI change±SE were: advocacy (16.3±2.0 vs 0.2±1.7); burden (9.9±1.3 vs 2.3±1.0); convenience (12.6±1.6 vs 1.2±1.1); efficacy (13.9±1.7 vs 2.9±1.5); flexibility (12.2±1.4 vs 1.6±1.3); general satisfaction (18.3±2.2 vs 0.3±1.6); pain (14.2±1.5 vs 0.8±1.2); preference (27.4±2.1 vs 1.7±1.5); side effects (3.4±1.2 vs -2.6±1.2), all p<0.0001; hassle (9.7±1.2 vs 4.4±1.2); life interference (9.8±1.2 vs 5.1±1.2); social (1.4±0.8 vs -0.9±0.7); and insulin-specific satisfaction (9.7±1.7 vs 0.8±1.1), all p<0.01 to 0.03. The overall quality-of-life scale and subscales of behavioral and emotional control, general and hyperglycemic symptom distress, overall cognition, mental acuity and awareness also improved more favorably for INH as compared to MDI, all p < 0.01 to 0.05. Treatment differences could not be completely explained by HbA_{1c} decreases from baseline [INH (0.3±0.06%) vs MDI (0.1±0.07%), p=0.08]. **Conclusions:** Reducing the number of injections by using inhaled insulin improves patient satisfaction and quality of life, thereby reducing therapeutic burden and adherence barriers to intensive therapy.

9

PULMONARY DELIVERY OF INSULIN USING THE AERx™ INSULIN DIABETES MANAGEMENT SYSTEM IN HEALTHY AND ASTHMATIC SUBJECTS.

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Background: The AERx™ iDMS (insulin Diabetes Management System) is a potential diabetes treatment option for delivery of a liquid aerosol of human insulin to the deep lung for systemic absorption. **Methods:** The PK, PD, and safety of pulmonary insulin were compared in 28 healthy (H) and 16 asthmatic (A) (FEV1 50-80%) subjects in a 3-period, open-label trial. The same single inhalation dose, equivalent to approximately 6 subcutaneous units of human insulin was given in the first two periods and an insulin dose equivalent to approximately 17 subcutaneous units was given in the third period to assess safety and pulmonary function. **Results:** Insulin AUC_(0-360 min) was significantly higher for healthy than for asthmatic subjects (p=0.013), whereas no difference was observed for C_{max} (see Table). Greater reduction in serum glucose (indicated by AOC_(0-360 min)) was observed in healthy subjects (p=0.007). No significant changes in FEV1, FVC, and FEV1/FVC were found from pre- to post-dose and no other safety issues arose. **Conclusions:** Asthmatic subjects absorbed less insulin than healthy subjects, resulting in less reduction of serum glucose. No effects on airway reactivity were observed, even when the higher dose was given.

Table. PK and PD Parameters of Pulmonary Insulin (Mean(SD))

Parameter	H (N=28)	A (N=16)	p value	H/A	90% C.I.
Insulin AUC ₍₀₋₃₆₀₎ (pM*min*kg)	1.4x10 ⁶ (7.5x10 ⁵)	1.1x10 ⁶ (6.4x10 ⁵)	0.013	1.58	1.13-2.21
Insulin C _{max} (pM*kg)	9.9x10 ³ (5.7x10 ³)	8.3x10 ³ (5.4x10 ³)	0.094	1.25	0.95-1.65
Glucose AOC ₍₀₋₃₆₀₎ (mg/dL*min)	4.9x10 ³ (2.2x10 ³)	3.4x10 ³ (1.7x10 ³)	0.007	1.40	1.12-1.74

10

IMPACT OF PARTICLE SIZE AND AEROSOLISATION TIME ON THE METABOLIC EFFECT OF AN INHALED INSULIN AEROSOL

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Background and Aims: We investigated the metabolic effect of inhaled insulin (INH) administered by the Aerodose™ Inhaler with 2 particle sizes (fine (F) vs. very fine (VF), both <3 µm, mass median aerodynamic diameter) and 2 aerosolisation times (drug release for the first 2 or 4s per 5s inhalation). **Materials and Methods:** Thirteen healthy volunteers (non-smokers, 31±6 years (mean±SD)) completed this euglycaemic glucose clamp study (180 min baseline, clamp level 5.0 mmol/L, continuous i.v. insulin infusion at 0.15 mU/kg/min). Subjects received 0.15 U/kg regular insulin s.c. (SC) on the first study day, and 1.5 U/kg INH (VF/2s, VF/4s, F/2s, or F/4s) on the following four study days. Glucose infusion rates (GIR) were registered for the subsequent 360 min. **Results:** The longer (4 vs. 2s) aerosolisation time resulted in significantly higher maximal metabolic action (GIR_{max}), total metabolic activity (AUC_{0-360min}) and relative biopotency (treatments VF/4s+F/4s vs. VF/2s+F/2s, p<0.05). The VF particle size showed higher values for AUC_{0-360min} and relative biopotency vs. the F particle size, but this did not reach statistical significance. Time to half-maximal action (early t_{GIR50%}) values were shorter for INH vs. SC (p<0.05, except for VF/2s). Time to maximal action (t_{GIRmax}) trended similarly (p<0.1 except for VF/2s). Aerosolisation time and particle size did not affect early t_{GIR50%} or t_{GIRmax}. No drug- or device-related adverse events were observed. **Conclusions:** Aerosolisation time, but not the difference in particle size studied, had a significant impact on the metabolic effect elicited by inhaled insulin, allowing rational selection of delivery parameters for subsequent trials. Based on the observed biopotency and rapid onset of action, the Aerodose Inhaler shows significant promise for covering prandial insulin requirements.

Treatment	SC	VF/2s	VF/4s	F/2s	F/4s
GIR _{max} (mg/kg/min)	8.0±2.7	7.2±2.4	8.4±2.6	6.6±2.4	8.1±3.6
Early t _{GIR50%} (min)	61±29	46±80	34±30	33±20	30±17
t _{GIRmax} (min)	175±69	158±91	132±72	127±54	128±55
AUC _{0-360min} (g/kg)	1.9±0.5	1.8±0.6	2.1±0.9	1.6±0.7	2.0±0.9
Biopotency (%)	-----	10±2	12±4	8±3	11±4

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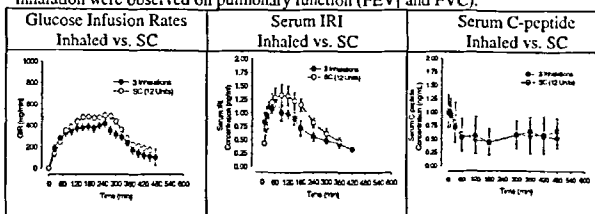
TIME-ACTION AND DOSE-RESPONSE PROFILES OF INHALED INSULIN USING THE SPIROS® DRY POWDER INHALER

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Aims: This study investigated the pharmacokinetics, glucodynamics, safety and tolerability of human insulin in healthy volunteers using the Spiros® dry powder inhaler (DPI), a breath-actuated, palm-sized pulmonary drug delivery system.

Materials and Methods: This was an open-label randomized crossover trial conducted in 26 healthy, 21 to 55 year-old, nonsmokers with normal pulmonary functions. Subjects were administered 2 doses of human insulin inhalation powder, chosen from 2, 3, 4 or 5 inhalations, and 2 doses of subcutaneous (SC) regular human insulin (U100), chosen from 8, 12, 16 or 20 U, on separate visits after a 10 hour fast. Total immunoreactive serum insulin (IRI), C-peptide, and glucose requirements following inhaled human insulin were evaluated during a euglycemic clamp procedure.

Results: Mean IRI and glucose requirements peaked earlier following inhalation than after SC insulin. The duration of action, glucose requirements, and dose-response relationships were comparable between the two routes of administration. Suppression of C-peptide was observed throughout the duration of insulin action. Both insulins were well tolerated. No clinically significant effects of insulin inhalation were observed on pulmonary function (FEV1 and FVC).



Conclusions: Oral inhalation of human insulin via the Spiros® DPI is a promising alternative method for administration of human insulin.

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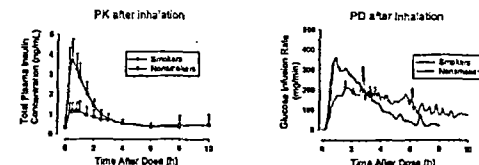
SMOKING INCREASES THE BIOAVAILABILITY OF INHALED INSULIN, BUT RELATIVE INSULIN RESISTANCE AMELIORATES DIFFERENCES IN ACTION

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Aims: Smoking may impact bioavailability of inhaled drugs. Previous studies have shown that smokers experience increased absorption of nebulized insulin. A study was performed with inhaled insulin administered by the Spiros DPI system to investigate impact of smoking on pharmacokinetic (PK) and pharmacodynamic (PD) profiles.

Materials and Methods: The euglycemic clamp was used to define the time-action profile of subcutaneous regular (SC) and human insulin inhalation powder (HIIP) in 2 groups of 8 healthy non-diabetic non-smokers (NS) and smokers (S) within an hour of cigarette use. A crossover design was used in which each subject received each treatment on 2 occasions. Doses were 12U SC and 2.31mg HIIP emitted from the inhaler. PK parameters were calculated from serum insulin vs time, PD parameters from glucose infusion rate vs time.

Results: Matched subjects 23±1.5yrs with BMI of 22.5±2kg/m². SC insulin profiles were consistent with historical data. Total insulin systemic exposure was greater in S and the parameter, G_{max}/AUC ratio was significantly lower for S following both routes of administration, suggesting relative insulin resistance.



Conclusion: Insulin absorption across the lung was enhanced in S. S effect on PD was less pronounced than PK. This may be related to relative insulin resistance ameliorating the response to greater peak insulin after inhalation in smokers. These factors should be considered in determining dosage regimens of inhaled insulin in smokers.

OP 3

Fat and Insulin Resistance

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BROWN ADIPOSE TISSUE-SPECIFIC INSULIN RECEPTOR KNOCKOUT MICE FED ON HIGH FAT DIET SHOW OBESITY AND DIABETES PHENOTYPE.

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Background and Aims: Type 2 diabetes (NIDDM) is a polygenic disease characterized by defects in both insulin secretion and insulin action. Among the insulin resistance candidate genes, insulin receptor (IR) has met a special clinical relevance. Mice with a global knockout of the IR die within 4-5 days after birth. However, several tissue-specific IR knockout mice are viable and show several phenotypes.

Material and Methods: We have generated mice with brown adipose tissue-specific insulin receptor knockout (BATIRKO), crossing mice expressing Cre under the uncoupling protein 1 (UCP-1) promoter and LoxP-LoxP-IR.

Results: At 6 months of age, BATIRKO mice had interscapular brown adipose tissue (IBAT) about 25% of normal size. However, the white fat mass/body weight ratio remained unaltered throughout development, under standard diet. Brown fat atrophy is accompanied by a significant decrease of b-cell mass (by 40%) and an severe insulin secretion defect, resulting in fasting hypoinsulinemia and hyperglycemia and also glucose intolerance in response to IP glucose, without hyperlipemia. However, no insulin resistance was found in BATIRKO mice. Male BATIRKO mice fed on standard diet (3 Kcal/g) for 23 weeks increased body weight by 25% versus controls (15%). However, male BATIRKO mice fed on high fat diet (4.8 Kcal/g) for 23 weeks significantly increased body weight by 36% versus controls (21%). Under high lipid diet, male BATIRKO mice show insulin resistance without a compensatory insulin secretion (hypoinsulinemia versus hyperinsulinemia in the controls), resulting in a severe glucose intolerance.

Conclusions: This model provides evidence for a novel role of brown fat in the regulation of insulin secretion and glucose homeostasis, and also in the body weight control.

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OVEREXPRESSION OF HORMONE-SENSITIVE LIPASE IN BETA-CELLS RESULTS IN BLUNTED GLUCOSE STIMULATED INSULIN SECRETION AFTER FEEDING MICE A HIGH-FAT DIET

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Background and Aims: Hormone-sensitive lipase (HSL) is expressed and active in pancreatic beta-cells. Since HSL hydrolyze intracellular triglycerides in adipose tissue, it is possible that HSL in beta-cells is involved in the production of a lipid-derived signal that seems necessary for optimal glucose stimulated insulin secretion (GSIS). The importance of HSL and lipids for beta-cell function was therefore examined using transgenic mice overexpressing HSL specifically in beta-cells. **Material and Methods:** Transgenic mice were generated using adipocyte HSL cDNA together with the rat insulin promoter II on a C57BL/6J genetic background. Several lines were identified and in this study, a line with 80-fold overexpression of HSL was phenotyped. Body weight and circulating glucose, insulin, leptin and free fatty acids (FFA) were examined in transgenic (tg) and wild-type littermates (wt) in the F2 generation. To study beta-cell performance, glucose (IVGTT, 1 g/kg) or arginine (0.25g/kg) was injected intravenously. Animals were fed either a standard diet or a high-fat (58%) diet. **Results:** There were no differences in basal circulating glucose, insulin, leptin or FFA during feeding the standard diet. IVGTT displayed no significant difference regarding glucose tolerance or insulin levels in tg mice. However, after feeding a high-fat diet for 1-2 months, tg mice showed impaired glucose tolerance with a blunted insulin response upon IVGTT, with an acute (1-5 min) insulin response (AIR) of -857 ± 385 pmol/l in tg males and 1714 ± 985 pmol/l in wt males ($p < 0.05$). The low insulin response was reflected by elevated plasma glucose (wt 33.3 ± 0.5 mmol/l, tg 37.4 ± 0.5 mmol/l ($p < 0.01$) at 5 min) throughout the 75 min experiment. After 3-4 months of high fat feeding tg mice were hyperglycemic (male wt 9.6 ± 0.6 mM, male tg 13.0 ± 0.8 mM, ($p < 0.05$), female wt 4.0 ± 0.3 mM, female tg 13.7 ± 1.9 , ($p < 0.05$)). Iv arginine resulted in a normal insulin response with no significant differences between wt and tg mice. **Conclusions:** Mice with 80-fold overexpression of HSL in beta-cells grow and develop normally. The mice displayed a normal GSIS when fed a normal chow, whereas when fed a high-fat diet, tg mice had a blunted insulin response compared to wt mice. This was associated with impaired glucose tolerance. In contrast, the insulin response to arginine challenge was not affected by HSL overexpression. The study therefore suggests that HSL and beta-cell lipids are of importance for the adaptive GSIS in insulin resistance.

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Insulin Regulation of Regional Lipolysis in Obese and Lean Humans.

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Background and Aims: Visceral obesity is associated with insulin resistance with respect to antilipolysis. Whether this insulin resistance is primarily located in visceral adipose tissue has not been determined.

Materials and Methods: We, therefore, measured systemic and regional palmitate release ([3H]palmitate) in lean men and women (BMI 23.7 ± 0.8 and 22.2 ± 0.9 kg/m²; visceral fat 88 ± 19 and 26 ± 5 cm²) and upper body obese men and women (BMI 32.7 ± 1.4 and 34.9 ± 0.7 kg/m²; visceral fat 214 ± 28 and 115 ± 15 cm²) (n=7 in each group) under basal conditions, during low-dose and high-dose insulin infusion using the euglycemic clamp technique.

Results: Basal palmitate flux was somewhat greater in obese men than lean men (158 ± 18 vs. 127 ± 17 μ mol/min, $P=0.12$), and greater in obese women than lean women (265 ± 29 vs. 177 ± 20 μ mol/min, $P<0.05$). A progressive suppression of palmitate flux was observed in each group with the higher insulin doses. During the high-dose insulin infusion palmitate release was greater in obese men than lean men (34 ± 6 vs. 20 ± 2 μ mol/min, $P<0.04$), and in obese women than lean women (45 ± 12 vs. 29 ± 8 μ mol/min, $P=0.15$). In the face of marked suppression of systemic palmitate release during the high-dose insulin infusion, there was a significant increase in the percent of systemic palmitate release that originated from the splanchnic bed in each group. There were no significant differences in the proportion of systemic palmitate originating from the splanchnic bed between groups (lean women $18 \pm 3\%$, obese women $30 \pm 13\%$, lean men $25 \pm 6\%$, obese men $24 \pm 2\%$), however. Leg palmitate release was not significantly different between groups, indicating that upper body nonsplanchnic adipose tissue remained the major difference between systemic FFA delivery in obese vs. lean humans.

Conclusions: Although there is insulin resistance with respect to suppression of lipolysis in upper body obesity, this does not primarily originate from visceral adipose tissue. Resistance to the antilipolytic effects of insulin in the upper body subcutaneous adipose tissue depot is the main source of elevated FFA in visceral obesity.

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SURPRISINGLY SEVERE INSULIN RESISTANCE IN THE ADIPOSE TISSUE OF HIV-INFECTED CHILDREN WITH LIPOHYPERTROPHY

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Background: The lipodystrophic syndrome, which associates redistribution of adipose tissue (AT) with insulin resistance and dyslipidaemia, prevails in HIV-infected children as in adults. The aim was to assess *in situ* insulin sensitivity of AT with lipohypertrophy (LH+) using the microdialysis technique in HIV-infected children.

Materials and Methods: Insulin sensitivity, assessed by the inhibition of glycerol release in the abdominal subcutaneous AT, was measured during OGTT in 5 children with abdominal HIV/LH+ (supra-iliac skinfold thickness $> 97^{\text{th}}$ perc.), in 5 healthy obese children matched for sex and pubertal stage, and in 10 HIV-infected children without lipodystrophy (HIV/LD-). **Results:** (mean \pm SD)

	Obese	HIV/LH+	HIV/LD-	p*
Age (years)	10.7 \pm 1.7	12.0 \pm 4.5	9.1 \pm 1.9	ns
Protease Inhibitor Treatment (n)	-	4	9	ns
CD4 (cells/mm ³)	-	691 \pm 441	805 \pm 329	ns
Supra-iliac skinfold thickness (mm)	33.7 \pm 6.4	19.3 \pm 15.4	6.9 \pm 2.4	0.001
Percent Fat Mass (%)	37.8 \pm 5.0	22.8 \pm 10.9	18.8 \pm 5.4	0.008
NGT during OGTT (n)	5	5	10	ns
Fasting Serum Insulin (μ U/ml)	8.0 \pm 3.2	6.2 \pm 4.5	2.6 \pm 1.5	0.009
Fasting Plasma Glycerol (μ mol/l)	106 \pm 25	59 \pm 38	72 \pm 34	ns
T ₁₂₀ -OGTT-Serum Insulin (μ U/ml)	54.2 \pm 37.1	54.7 \pm 47.7	19.1 \pm 9.1	0.049
Dialysate Ratio Glycerol T ₁₂₀ -T ₀ / Glycerol T ₀ (%)	-44 \pm 11	-41 \pm 12	-54 \pm 17	ns
% Δ Glycerol T ₁₂₀ -T ₀ / Insulin T ₁₂₀	-1.2 \pm 0.8	-1.1 \pm 0.7	-3.4 \pm 2.1	0.008

*: p values for non-parametric tests (3 groups)

In situ insulin resistance of AT was measured as high in HIV/LH+ as in obese children despite a two-fold less skinfold thickness and basal lipolytic activity. **Conclusions:** These data argue in favour of profound changes not only in the morphology but also in the function of adipose tissue in lipodystrophies associated with HIV infection.

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Effects of short term dietary intervention on intramyocellular lipid content (IMCL) and Insulin Sensitivity (IS)

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Background and Aims: An increased IMCL content as quantified by ¹H-MR-spectroscopy (MRS) has been found to be associated with reduced insulin sensitivity. These IMCL seem to be rapidly regulated as one single bout of exercise decreases IMCL, while the combination of hyperinsulinemia and elevated circulating levels of free fatty acids increases IMCL within 4 hours. A high fat intake has been found to be associated with high risk for type 2 diabetes mellitus. Bessesen et al recently showed, that a major part of orally given labelled ¹⁴C oleate is stored in the skeletal muscle as TG, indicating an important role of skeletal muscle in the trafficking of dietary fat.

Materials and Methods: To observe the effects of a short term dietary intervention (3 days) on IMCL and IS we examined 12 male, healthy, lean subjects. After three days of a standard mixed diet, IMCL was quantified by ¹H-MRS after an overnight fast in the red soleus muscle and the mixed tibialis anterior muscle. In addition IS was quantified by euglycemic-hyperinsulinemic glucose clamp (GIR ml/min kg). The measurements were done at baseline and after three days of a hypercaloric diet (HC: 57,6% fat; 32,3 % CHO; 13 % protein; 2782 kcal/day) and at baseline and after three days of normocaloric diet (NC: 21 % fat; 63,3 % CHO, 16,7 % protein; 2015 kcal/day). Results are presented in % change from baseline.

Results: IMCL varied over a wide range. After three days of hyper caloric diet IMCL was significantly (*p<0,05) increased in the tibialis ant. muscle (+48,0%), while IS was significantly (*p<0,05) decreased by 16,7%. In contrast we observed no relevant changes in IMCL and IS after three days of a normocaloric diet.

Conclusions: Therefore these data indicate that a hypercaloric diet decreases IS and profoundly influences the formation of IMCL.

	After 3 days of NC	After 3 days HC
IMCL soleus (%)	+7,8	+14,4
IMCL tibialis ant.(%)	-10,1	+48,0*
GIR (%)	+3,2	-16,7*

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Normalisation of muscle triglycerides stores and insulin resistance by L-Arginine in sucrose-fed rats.

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Background and Aims: Intracellular triglyceride (TG) is an important energy source for skeletal muscle. However, recent evidences suggest that elevated tissue TG stores are strongly associated with insulin resistance. Moreover, impaired nitric oxide activity and endothelial dysfunction have been demonstrated in the presence of increased TG levels. Aim of the study was to evaluate the effects of L-arginine, a precursor of nitric oxide, on muscle TG stores and metabolic parameters in a rat model of insulin resistance.

Materials and Methods: Experiments were performed on male Sprague-Dowley rats. All rats were fed for 1 week with a control chow (CT) diet. After this period of acclimatization, 16 rats continued on the CT diet, 12 rats were switched to a sucrose-enriched (SU) diet, containing 68% sucrose, 20% protein, 12% lipid and 8 rats were switched to a sucrose plus L-arginine (1.25 gr/day) enriched diet (SU+ARG), for 8 weeks. Fasting samples were withdrawn for the assay of metabolic parameters while intracellular TG were measured on soleus muscles by Gas Chromatography and Mass-Spectrometry.

Results: After 8 weeks of SU diet, rats presented all the metabolic features of the Insulin Resistance Syndrome since significant increases of body weight (519±8 vs 388±2 g; p<0.0001; SU vs CT respectively), retroperitoneal fat (23±1 vs 6±1 g; p<0.0001), blood glucose (109±29 vs 69±1 mg/dl; p<0.05), serum insulin (173±62 vs 46±10 pmol/l; p<0.05), serum triglycerides (116±22 vs 53±4 mg/dl; p<0.005) and nitric oxide levels (8.5±0.8 vs 3.0±0.2 µmol/l; p<0.0001) were demonstrated. In addition, a three fold increase of intramuscular TG was observed (2510±557 vs 876±77 nmol/ g tissue; p<0.0033). SU+ARG diet normalised intramuscular TG (749±142 nmol/ g tissue), serum TG (44±13 mg/dl), blood glucose (76±5 mg/dl) and serum insulin (50±8 pmol/l). Moreover, a significant decrease of body weight (478±7 g), retroperitoneal fat (15±2 g) and nitric oxide (6.2±0.2 µmol/l) was observed.

Conclusions: L-arginine is able to normalise muscle TG stores and to revert insulin resistance in sucrose-fed rats and might be an useful tool in the treatment of Insulin Resistance Syndrome.

OP 4

Epidemiology – Type 2 Diabetes

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Epidemic Glucose Intolerance in Australia.

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Background and Aims: Diabetes is a growing public health problem around the world. However, there are few national population based prevalence data, especially in developed countries. The Australian Diabetes, Obesity and Lifestyle (AusDiab) study was carried out to provide the first ever national estimates of the prevalence of diabetes and other states of glucose intolerance in Australia.

Materials and Methods: Adults aged 25 and over residing in 42 randomly selected cluster areas across the country were invited to participate between May 1999 and December 2000. Participants attended a local survey site for a physical examination including a standard 2-hour 75g-g oral glucose tolerance test. Classifications were made using 1999 WHO diagnostic criteria. Results were weighted to the Australian 1998 population, using census data.

Results: 11,247 adults took part, representing a 55.3% response rate of the total eligible population. The prevalence of diabetes was 7.5% (men – 8.0%, women – 7.0%). The prevalence rose from 0.3% among those aged 25-34 to 23.6% in those aged 75 and over. The prevalence of undiagnosed diabetes (3.8%) was as high as that of previously diagnosed diabetes (3.8%). Impaired glucose tolerance was more common in women than men (12.0% vs 9.2%), but impaired fasting glucose was more common in men than women (8.1% vs 3.3%). In total, 23.8% (men – 25.3%, women – 22.3%) of Australians aged 25 years and over had diabetes or impaired glucose metabolism.

Conclusions: This study shows that almost 1 in 4 Australian adults have diabetes or impaired glucose metabolism. This is one of the highest recorded prevalences in the developed world, and indicates the urgent need to address the relevant public health issues.

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It is possible to reduce the number of OGTT by 60% using a stepwise screening strategy combining HbA1c and fasting plasma glucose compared to WHO's screening strategy.

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Background and Aims: The prevalence of type 2 diabetes is increasing in Denmark. The proportion of undiagnosed type 2 diabetes in the younger age groups is 2/3 of all individuals with diabetes; this indicates the need for screening of the disease. WHO recommend that all individuals with impaired fasting glucose (IFG) undergo an OGTT. The oral glucose tolerance test (OGTT) is inconvenient as a screening method. It has a poor reproducibility, the persons have to be fasting, and it is time consuming. The purpose of this abstract is to develop a screening model combining HbA1c and fasting plasma glucose that can reduce the use of OGTT compared to the stepwise screening strategy recommended of WHO.

Materials and Methods: A population-based sample of 13.000 individuals aged 30 to 60 years living in Copenhagen County are invited. This abstract is based on preliminary results based on 3225 participants (139 newly diagnosed individuals with diabetes (NDM), 3086 with normal glucose tolerance (NGT), individuals with known diabetes was excluded).

Results: The sensitivity and specificity of WHO's stepwise screening strategy are 79, 100% respectively.

We have developed a model where the first step is HbA1c ≥5.6 % (75% of the population) subsequently the fasting plasma glucose dividing individuals in NGT (FPG<6.1 mmol/l), DM (FPG≥ 7.0mmol/l), and IFG (FPG6.1-6.9mmol/l). Finally Individuals with IFG are combined with HbA1c ≥ 6.1%. The sensitivity and specificity are 71and 94% respectively. This model reduces the need of OGTT from 416 to 201 (12% - 6% in the population).

Conclusions: HbA1c is an easy method; the individual does not have to be fasting. Using a stepwise screening where the HbA1c as the first step reduces the number of individuals that have to be fasting with 25%. It is possible to reduce the number of OGTT with 50% by using a stepwise screening strategy including HbA1c and FPG in special combinations. It is likely that the sensitivity will increase if this model is implemented in a high risk population detected by a questionnaire.

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GROWTH IN INFANCY AND CHILDHOOD AND TYPE 2 DIABETES IN ADULT LIFE

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Background and Aims: Low birth weight is associated with increased rates of type 2 diabetes in later life. This is thought to be a consequence of persisting physiological and metabolic changes that accompany slow growth in utero. Furthermore, both childhood and adult obesity are important risk factors for type 2 diabetes. The aim of this study was to examine growth during infancy and early childhood among persons who develop type 2 diabetes.

Materials and Methods: Men (n=4630) and women (n=4130) who were born in the Helsinki University Central Hospital during 1934-44, and who attended child welfare clinics in the city participated in the study. Each individual had on average 18 measurements of height and weight between birth and twelve years of age. Detailed birth and child welfare records were available for all 8760 participants.

Results: We identified 208 men and women who developed type 2 diabetes. Low birth weight and low ponderal index (birthweight/length³) were associated with increased risk of type 2 diabetes. Low weight (p=0.019), and body mass index at two years of age (p<0.001) also increased the risk. After the age of 7 years rapid gain in weight and body mass index increased the risk of type 2 diabetes. The cumulative incidence of type 2 diabetes was positively and strongly associated with BMI at age 11 years (p<0.001) among both men and women.

Conclusions: This study shows that, irrespective of size at birth, low weight gain during infancy is also associated with increased risk of the disease. Furthermore, after 7 years of age, rapid weight gain and higher BMI in childhood is associated with further increase in risk of type 2 diabetes.

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Glucose Metabolism, Adiposity and Fitness interrelations in a Biracial Sedentary Population: The HERITAGE Family Study

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BACKGROUND AND AIMS: defects in the secretion of insulin and resistance to its actions both play a role in the pathogenesis of diabetes mellitus. It is therefore important to identify the factors that contribute to their development. **METHODS:** Multicenter family study, 259 Blacks and 492 Whites measured for body mass index (BMI), abdominal CT-scan visceral (AVF) fat area, maximal oxygen uptake (VO₂max), glucose induced insulin secretion (IA10), insulin sensitivity (SI), glucose disappearance index (Kg) and disposition index (DISP) derived from Minimal Model analysis of intravenous glucose tolerance tests. **RESULTS:** Men had lower unadjusted Kg (p=0.0001), SI (p=0.025) and DISP (p=0.002) than women. Blacks had lower SI (p=0.0001) but higher Kg (p=0.0001), DISP (p=0.0001) and IA10 (p=0.0001) than Whites. Parents had lower Kg (p=0.0001), IA10 (p=0.0001) and DISP (p=0.0001) than offspring. Stepwise multiple regression analysis determined that the independently significant covariates were, in order of importance, age, sex, race and BMI for Kg; BMI, race, AVF, age, VO₂max, sex and generation for SI; race, age, AVF, sex, VO₂max and BMI for IA10; AVF, race, age and BMI for DISP. After adding metabolic phenotypes in the regression model, the main covariate for Kg was DISP, partial-R²: 0.63, p=0.0001 (model R²: 0.65). After adjusting for these covariates, differences between genders were maintained for SI (p=0.0001) and became significant for IA10 (higher in men, p=0.0001), all those between races remained significant (p=0.0001-0.0008), and only SI was different between generations (greater in offspring, p=0.015). **CONCLUSIONS:** These results suggest that Blacks and Whites differ in glucose metabolism phenotypes, possibly through race-specific environmental and genetic elements. Gender-specific factors may explain the lower insulin sensitivity and higher insulin secretion in men, and age, the difference in insulin sensitivity between parents and offspring. Moreover, the disposition index appears to be the main determinant of intravenous glucose tolerance, as measured by Kg.

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IDENTIFICATION OF SUBJECTS WITH ISOLATED POSTLOAD HYPERGLYCAEMIA. THE HOORN STUDY

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Background: There is ample evidence that the postload glucose level (2 hours after a 75- g oral glucose tolerance test) is a better predictor for mortality than fasting glucose. Because in clinical practice glucose testing is not feasible, fasting glucose is used for the diagnosis of diabetes and impaired glucose tolerance is not detected. Consequently, about half of the persons with chronic hyperglycaemia and elevated risk will be missed. We studied strategies for the identification of persons with elevated postload glucose and non-diabetic fasting glucose.

Methods: In the Hoorn Study, a population based study, 2259 subjects aged 50-75 without known diabetes and fasting glucose < 7.0 mmol/l had a 75 g glucose tolerance test in 1989-1990. Hb1c and other cardiovascular risk factors were determined. Isolated postload hyperglycaemia (IPH) was defined as fasting glucose < 7.0 and postload glucose ≥ 7.8 mmol/l. Impaired Fasting glucose (IFG) was defined as fasting glucose 6.1-7.0 mmol/l. Logistic regression analysis with was used to study characteristics which contribute to the prediction of IPH.

Results: A total of 274 (12%) persons had IPH. Of these, 33 % had IFG vs 10 % of persons without IPH. Other less important but significant predictors of IPH were: age >65, HbA1c ≥ 6.5, hypertension (systolic pressure ≥ 160, diastolic pressure ≥ 90 or medication), BMI ≥ 30, and HDL cholesterol < 1. Having ≥ 2 of these risk factors identified 162 of the 274 (59%) with IPH. However, also 527 (27 %) of 1985 persons with normal postload levels had ≥ 2 of these risk factors.

Conclusions: Persons with isolated postload glucose were characterised by IFG, elevated HbA1c, hypertension, overweight, and low HDL. IFG and high HbA1c alone or in combination with other cardiovascular risk factor did not result in a sensitive or specific strategy to identify IPH. However, the identified persons are those who may benefit from treatment of cardiovascular risk factors.

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Hyperinsulinaemia as long-term predictor of death and heart disease in non-diabetic men

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Background and Aims: Prospective studies have indicated that hyperinsulinaemia (HI) is a risk factor for ischaemic heart disease (IHD), risk decreasing with time of follow-up. Few studies have so far investigated the role of HI for the prediction of mortality.

Materials and Methods: In 6074 non-diabetic Swedish males (mean age 47 years), free of known cardiovascular disease and type 2 diabetes, we determined IHD risk factors including blood glucose and serum insulin before and after 2 hours after an OGTT. Follow-up was 19 years for IHD and death divided into three periods - 6, 12 and 19 years. HI was defined as values above the 10th decile of fasting or 2-hour insulin levels.

Results: Relative Risks (RR:s) for both death and IHD were J-shaped when subjects were divided into deciles of fasting insulin with the highest RR (1.4) in the HI group. The differences between groups in RR:s for death and IHD events decreased after adjustment for risk factors and after longer follow-up. The risk of death in HI men, defined on the basis of 2-hour insulin level increased with time of follow-up and was significantly increased after 12 years, RR 1.41 (95%CI: 1.00-2.00), and 19 years, RR 1.31 (1.05-1.65), even after full adjustment for possible confounders. The same pattern was found for IHD, but the difference was not statistically significant after adjustment for BMI.

Conclusions: Hyperinsulinaemia is a risk factor for death and IHD events in non-diabetic men although risk decreases after adjustment for traditional risk factors and with time. This supports the role of HI - a marker of insulin resistance - as an important cardiovascular risk factor. The finding of different pattern of risk versus time of follow-up for fasting and 2-hour insulin calls for further research. Intervention studies based on life style or drugs that improve insulin sensitivity are urgently needed to prove or disprove the causality of this association.

OP 5 Experimental Immunology

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THE HUMAN DQ8 HLA CLASS II MOLECULE PRESENTS DIABETOGENIC T CELL EPITOPES OF THE PHOGRIN ANTIGEN

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Background and Aims: The protein tyrosine phosphatases IA-2 and Phogrin (IA-2 beta) are major autoantigens in Type 1 diabetes. We have previously demonstrated that Phogrin-specific T cell clones generated from NOD mice are capable of destroying pancreatic islets and cause diabetes in vivo. We identified two distinct T cell epitopes on the Phogrin antigen defined by amino acids 640-659 (Peptide 2) and 755-777 (Peptide 7). The aim of our study was to examine whether the Phogrin epitope peptides are compatible with the human DQ8 HLA Class II molecule, an allele associated with high susceptibility to Type 1 diabetes in humans. **Materials and Methods:** DQ8+ transgenic mice (transgenic animals expressing the human DQ8 HLA Class II gene without any mouse Class II allele expressed) were immunized with Phogrin epitope peptides Peptide 2 and Peptide 7 respectively, in Complete Freund's Adjuvant. A Peptide 2 specific T cell line was fused with BW5147 lymphoma cell line and hybrid clones were derived with limiting dilutions. **Results:** Lymph node cell populations of the immunized DQ8+ animals displayed vigorous T cell proliferative responses to both of the Phogrin epitope peptides. DQ8+ T cell lines, generated with serial stimulations with the specific peptide retained the strong proliferative responses. Limiting dilutions of a responsive T cell hybrid line resulted in 11 Peptide 2 specific hybrid clones. All of the 11 T cell hybrid clones produced significant amounts of IL-2 in presence of Peptide 2 and DQ8+ spleen cells but not in the presence of Balb/c or NOD spleen cells. Alanine substitutions of Peptide 2 at three different residues (position 7, 10 and 14) ablated the IL-2 production in all clones, suggesting that these positions represent core binding residues between the DQ8 and the peptide. **Conclusions:** Both of the two distinct T cell epitopes of the Phogrin antigen are presented in the context of the human DQ8 HLA Class II molecule. We showed that the positions 7, 10 and 14 of Peptide 2 epitope represent core binding residues in the context of the DQ8 molecule. Our results suggest that the same two Phogrin epitopes might be represented in human Type 1 diabetes especially in patients with DQ8 HLA Class II background.

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IMPLICATION OF ADHESION MOLECULES IN PREVENTION OF DIABETES AFTER ORAL ADMINISTRATION OF CTB-INSULIN

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Background and aims: Immune tolerance towards an antigen can be obtained after oral administration. Minute amounts of insulin conjugated to B subunit of cholera toxin (CTB) induced protection of NOD mice from diabetes. Since adhesion molecules trigger the generation and homing of T cells from intestinal mucosa to peripheral lymph nodes, we investigated the role of L-selectin (CD62L) and $\alpha 4 \beta 7$ integrin in oral tolerance with CTB-insulin. **Materials and methods:** Cell phenotypes were analysed by FACS. Cytokine profiles were performed on activated cell supernatants by ELISA and on mRNA by RT-PCR. To evaluate the role of adhesion molecules, we cotransferred irradiated recipient mice with diabetogenic T cells and CD4+CD62L+ subpopulations from the spleen of fed mice. In addition, donor mice and recipient mice were successively treated with anti-CD62L or anti- $\alpha 4 \beta 7$ antibodies. **Results:** Oral administration of $2 \times 2 \mu\text{g}$ of CTB-insulin induced a significant increase of splenic CD4+CD62L+ cells in comparison to CTB administration ($38.5 \pm 8.8\%$ vs $32.1 \pm 7.1\%$, $p=0.01$). Analysis of cytokines indicated lower amounts of IL-4, IL-10 and IFN- γ in CD4+CD62L+ cells in comparison to CD4+CD62L- and significantly lower amounts of IFN- γ in CD4+CD62L+ cells derived from CTB-insulin fed mice. In contrast to CD4+CD62L- cells, transfer of CD4+CD62L+ cells derived from CTB-insulin fed mice reduced the capacity of diabetogenic T cells to transfer diabetes to syngeneic irradiated NOD mice (4/12 vs 8/8, 52 days post-transfer, $p<0.001$) and the degree of insulinitis in the recipients. This protective effect was inhibited by anti-CD62L antibody administration in the recipient mice, but was preserved despite anti- $\alpha 4 \beta 7$ antibody. Administration of either antibody to fed mice suppressed the capacity of T cells to transfer protection against diabetes transfer. **Conclusions:** These results suggest that oral tolerance induced by CTB-insulin administration implies CD4+CD62L+ regulatory cells. While both L-selectin and $\alpha 4 \beta 7$ integrin are required for the generation of regulatory cells, only L-selectin is necessary for their migration to pancreatic islets.

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SPECIFIC MODULATION OF PROCESSING AND PRESENTATION OF GAD65 BY B CELL GAD65 EPITOPES

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Background and aims: Autoantibody and T cell reactivity to glutamic acid decarboxylase-65 (GAD65) are common in type 1 diabetes. Whilst the disease is considered to be T cell dependent, the role of autoantibodies remains unclear. We have studied the role of antigen specific B cells in modulating the presentation of a dominant DRB1*0401 restricted T cell determinant of GAD65 in a cognate human autoreactive B-T cell system. **Materials and Methods:** 3 B cell clones, DPA, DPC, DPD, derived from a HLA DR3/DR4 new onset type 1 diabetic patient, have surface IgG on different determinants of GAD65. T cell line 6/7, derived from a HLA DR3/DR4 new onset type 1 diabetic patient, is specific for GAD65 residues 270-283. Stimulation of T cell line 6/7 was elicited by 0.1 to 10 $\mu\text{g/ml}$ recombinant human GAD65 or synthetic peptides of GAD65, 5G1(residues 266-285) or 16C2(residues 270-283). For proliferation assay, fully rested T cells (1.5×10^4) were added to 7.5×10^4 prepulsed, irradiated HLA-matched PBMC as APC. For presentation by the B cell clones, 1.5×10^4 rested T cells were cultured with 1×10^4 prepulsed irradiated B cells. Cell phenotypes were analyzed by FACS. Antibody affinity was determined by surface plasmon resonance on BIAcore. **Results:** Surface IgG on B cell clones (DPA, DPD) which recognize epitopes on structural domains of GAD65 different from the region harbouring the T cell epitope, efficiently ($\times 100\text{fold}$) enhance the presentation of residues 270-283, whilst B cell clone (DPC) the antibody of which overlaps the T cell determinant decreases presentation. These effects of B cell clones are dependent on surface IgG capture and internalisation of the antigen since they were inhibited by anti-IgG or chloroquine, and they are not dependent on autoantibody affinity ($K_d: 10^{10}\text{M}^{-1}$ for each). **Conclusions:** The data show that GAD65 autoantibodies may modulate T cell presentation in correlation with their topography on the structural model of GAD65, which may significantly shape the autoreactive T cell repertoire in type 1 diabetes.

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TCR-mediated Up-regulation of c-FLIPshort Correlates with Resistance to Fas-mediated Apoptosis in T-cells from patients with Type 1 Diabetes.

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Background and Aims: TCR activation leads to activation-induced cell death (AICD) that is an efficient way to remove T lymphocytes via apoptosis. We have previously showed that T cells from patients with Type 1 diabetes (T1-DM) are resistant to Fas-mediated apoptosis. In this study we investigated the molecular basis of apoptosis resistance upon reactivation in peripheral T lymphocytes from newly diagnosed T1DM patients (n=25) and age-matched normal controls (n=15).

Materials and Methods: Resting T cells (day 0) were cultured at 2×10^6 cells/ml with 1 mg/ml PHA for 16 h (day 1). Day 1 cells were cultured for an additional 4 days in the presence of 25 U/ml IL-2 (day 5). Day 5 pre-activated T cells were activated/reactivated via TCR/CD3 with plate coated anti-CD3 Ab OKT3 (30 mg/ml). Costimulation via CD28 was achieved by the addition of an anti-CD28 Ab (1.5 mg/ml) in addition to OKT3 for the same time period.

Results: Our results showed that PHA-stimulated day 1-cells from controls up-regulated Fas while Fas-expression was impaired in T cells from patients. In addition, T1-DM T cells remained resistant to Fas-mediated apoptosis during all time of culture period. The restimulation of activated T cells (day 5) via TCR/CD3 completely inhibited Fas-mediated apoptosis in T1DM vs control T cells ($p<0.001$). Costimulation via CD28 further reduced TCR/CD3-mediated apoptosis. After Fas triggering, generation of active sub-units of caspase-8 was significantly reduced in T1DM T cells restimulated via TCR/CD3 and/or CD28. We found that the level of c-FLIP and the cleavage product of c-FLIP in the DISC (Death Inducing Signaling Complex) were only slightly increased in both DM1 and normal T-cells. In contrast, the amount of c-FLIPs protein was significantly increased in the DISC only in T1-DM T cells restimulated via TCR/CD3 and via CD28 (4800 vs 1560 D.U.; 6353 vs 2300 D.U., respectively, $p<0.001$).

Conclusions: We conclude that the presence of increased levels of c-FLIPs prevents recruitment of pro-caspase -8 in T1-DM CD3-treated T cells. The high amounts of c-FLIPs are more likely to be involved in the inhibition of Fas-mediated apoptosis in T1-DM T cells. These data suggest that the recruitment of c-FLIPs into the DISC results in reduced DISC and caspase-8 activity in T1-DM and helps explaining the inability of peripheral autoreactive T cells elimination through apoptosis in T1-DM patients.

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RAT CYTOMEGALOVIRUS INFECTION ACCELERATES THE ONSET OF DIABETES IN DIABETES PRONE BB RATS

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Background and Aims: Viral infections, including cytomegalovirus (CMV), are thought to be involved in triggering the autoimmune process resulting in IDDM. Causal relationships between CMV infection and IDDM has not been shown so far and relevant animal models are lacking. Therefore, we analysed whether CMV infection influences the diabetogenic process using Rat Cytomegalovirus (RCMV) in a well established model for the development of autoimmune diabetes in rats.

Materials and Methods: Male and female DP BB rats (RT-1u) were divided in 6 groups. Groups 1, 2, 3 & 4 were infected with 5x10⁵, 1x10⁶, 2x10⁶ and 1x10⁷ plaque forming units (pfu) RCMV (Maastricht strain) respectively, at the age of 30-35 days. Groups 5 & 6 were control groups and were mock-infected or left untreated, respectively. Blood glucose levels were monitored twice a week, and animals were considered diabetic when glucose levels exceeded 16 mM. Diabetic animals were sacrificed and salivary glands and pancreas were taken out and processed for histological and PCR analysis. Presence of RCMV was analysed by immunohistochemistry (mAb8) and destruction of pancreatic islets was confirmed by HE and Aldehyde-Fuchsin (AF) staining.

Results: RCMV infection resulted in significant acceleration (2-3 weeks) of the onset of diabetes in all groups (groups 1-4) compared to the control groups (p = .0043). No differences between the different virus dosages were observed, and also both control groups showed no differences. Immunohistochemistry revealed that RCMV was only present in salivary glands and not in pancreatic tissue of infected animals at time of sacrifice, suggesting induction of diabetes without direct cytolytic infection of pancreatic beta-cells. This is strengthened by the observation that RCMV does not induce diabetes in diabetes-resistant BB-DR rats. Pancreatic tissue of RCMV infected BB-DP rats contained RCMV DNA as indicated by specific PCR analysis. Diabetic animals showed severe infiltration of the pancreatic islets and decreased AF staining, indicating beta-cell destruction.

Conclusions: This study shows for the first time a direct effect of CMV infection on the development of autoimmune diabetes in rats susceptible to spontaneous diabetes, without cytolytic infection of pancreatic beta-cells. These results enable us to further investigate the mechanism of virus-induced autoimmune diabetes.

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TAURINE GIVEN IN EARLY LIFE DECREASES INSULINITIS AND DELAYS THE ONSET OF DIABETES IN THE NOD

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Background : In human type 1 diabetes, insulinitis precedes β cell destruction. In spontaneous non-obese diabetic mouse (NOD), 80% of the females feature also insulinitis at 5-6 weeks of life, but less so in the males. We have previously reported a reduced β cell mass in neonatal female NOD mice preceding insulinitis. In the fetal and neonatal rat, the reduced and altered β cell mass induced by protein restriction during gestation and lactation is reversed by supplementation with taurine (a non-essential amino-acid important in fetal development). **Aims:** To study the effects of taurine supplementation early in life on the development of the endocrine pancreas, on insulinitis, and on the onset of diabetes in the NOD mouse. **Material and Methods:** Female NOD mice were supplemented with 2.5% taurine from day 1 of gestation until weaning. Controls NOD were given a normal diet. Pups were weaned on a normal diet, separated by sex and sacrificed at 8 weeks of age, or at the onset of diabetes. Pancreata were collected and fixed for histology. Islet area and scoring for insulinitis were performed in the pancreas at 8 weeks using a computerized image analysis system. **Results:** Blood glucose was 4-5 mM in both groups until 8 weeks of age but incidence of insulinitis was significantly reduced (7% vs 35%, (P< 0.0001) and islet area was increased in the taurine treated females. Islet proliferating cell nuclear antigen labelling was 4.7 \pm 0.3% (mean \pm sem) in the control female islets and 13.6 \pm 1.6% in the taurine treated. In ducts, a two fold increase in cell proliferation was found in the taurine treated group, although cell apoptosis did not differ between the groups. The percentage of β cells was increased in the taurine treated pups. Moreover, the mean onset of diabetes was 22 weeks (n=18) in the taurine treated NOD females compared to 14 weeks in the controls (n=8) (P<0.0001). **Conclusions:** These results suggest that supplementation with taurine during pregnancy and lactation had a beneficial effect in the NOD females as it reduced insulinitis, increased islet area and delayed the onset of diabetes.

OP 6

Cell Biology of the Endothelium

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Prevention of diabetes-induced capillary rarefaction of limb skeletal muscle by angiogenesis gene therapy with human tissue kallikrein

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Background and Aims: Peripheral vascular disease is very common among diabetic patients and consequences of supervening vascular occlusion are worsened by concomitance of microvascular rarefaction. We evaluated if human tissue kallikrein (HK) gene can prevent or rescue diabetic microangiopathy at the level of hindlimb skeletal muscle.

Materials and Methods: For prevention, the left adductor skeletal muscle of streptozotocin-induced diabetic mice was injected with an adenoviral vector containing the HK or the Luc gene, or saline 2 weeks after induction of diabetes. Animals were sacrificed at 28, 42, 60, 90, and 120 days for determination of adductor capillary density. In rescue experiments, gene therapy was applied 60 days after induction of diabetes and capillary density was determined 2 weeks later. Age-matched non-diabetic mice served as controls.

Results: After an initial increase in vascularity, untreated diabetic mice developed progressive microvascular rarefaction in hindlimb skeletal muscle. At 60 days, capillary density was reduced to 193 \pm 17 cap/mm², a value 3 times less (P<0.001) than that of non-diabetic controls (655 \pm 31 cap/mm²). Apoptosis was detected at the level of vascular endothelial cells, smooth muscle cells, and adventitial cells and, to a less extent, in the interstitium. The vasodilator response to acetylcholine was reduced, indicative of endothelial dysfunction. HK prevented diabetes-induced vascular rarefaction. Capillary density in HK-injected adductor was augmented to 760 \pm 85 and 1275 \pm 242 cap/mm² at 28 and 60 days, respectively, while no change was observed in contralateral adductor. Increased vascularity implies activation of cellular proliferation, however HK-injected muscles also showed reduced apoptosis at vascular level. Application of HK gene therapy at a later stage with the attempt of rescuing established microangiopathy also resulted in marked increase in capillary density. In contrast, insulin, although able to prevent endothelial dysfunction, neither altered the development of vascular rarefaction nor exerted additive microvascular effects in HK-treated mice.

Conclusions: These results indicate that kallikrein-kinin system may represent a new target for treatment of diabetes-induced peripheral microvascular disease.

Supported by a grant of the Juvenile Diabetes Foundation (JDF, USA).

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A NEW MODEL TO STUDY ANGIOGENESIS IN HYPERGLYCAEMIC CONDITIONS : THE DIABETIC CHICK EMBRYO.

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Background and aims: Diabetes makes ambiguous effects on angiogenesis, since neovessel sprouts occur in diabetic retina, while a reduced number of collateral vessels is observed in diabetic coronary vessels with atherosclerosis. We set up a new model to study the effect of hyperglycaemia on angiogenesis in the chorioallantoic membrane (CAM) in chicken embryos. **Materials and methods:** Fertilised White Leghorn chicken eggs were incubated in a humidified atmosphere at 37 \pm 0.5 C. Hyperglycaemia was induced by a single, intra-vitellus, 5 mg/g, glucose (G) injection at day 7 post-incubation. Control animals received mannitol (M), matched for osmolality or water (H). **Results:** Survival was 75 % (n=92) 7 days post-injection, i. e. at day 14 of incubation. Blood samples were drawn from a CAM vessel and blood glucose level was measured with a reflectancemeter. Mean blood glucose levels were increased up to 7 days post-injection (table, mean values (standard deviation)).

Day post inj.	Blood glucose level (mg/dl)						
	1	2	3	4	5	6	7
Glucose	192 (33)	176 (34)	214 (74)	173 (53)	162 (61)	155 (36)	157 (72)
Mannitol	109 (19)	95 (39)	84 (23)	117 (45)	90 (26)	101 (38)	100 (56)
Water	94 (8)	101 (19)	105 (21)	108 (14)	109 (22)	90 (3)	75 (15)

By ANOVA we found a significant treatment effect : G (n=195) vs M (n=93) and G vs H (n=60), P<10⁻⁴, M vs H, P= 0.51. Hyperglycaemic animals had severe growth delay. By day 7 post-injection, the mean body mass of embryos was 5.4 \pm 0.5 g (n=18) in glucose-injected animals, as compared to 9.8 \pm 1.9 g (n=16) and 8.0 \pm 1.1 g (n=18) in water- and mannitol-injected embryos, respectively. In hyperglycaemic embryos, CAM vessels were thinner, with decreased branching, and by day 7 post-injection, their number was reduced by 50%, as compared to water-treated control animals. Immunohistochemistry using anti-insulin and anti-glucagon antibodies showed no major difference in the numbers and proportions of pancreatic α and β cells between hyperglycaemic and control animals. **Conclusion:** This new model represents an easy alternative to previous models to analyse the molecular and cellular effects of hyperglycaemia on the blood vessel walls and angiogenesis.

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FREE FATTY ACIDS MODULATE ENDOTHELIAL APOPTOSIS, PROLIFERATION AND ASSOCIATED GENE/PROTEIN EXPRESSION

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Background and Aims: Elevated plasma free fatty acids (FFAs), as observed in the diabetic state, represent a risk factor for the development of premature atherosclerosis. Thus, it was the aim of the present study to evaluate the effects of saturated as well as unsaturated FFAs in cultured vascular endothelial cells. **Materials and Methods:** In human umbilical vein endothelial cells (HUVECs) apoptosis, proliferation and associated gene/protein expression in the presence of various FFAs (100-300 μ Mol/l; 24-48h) were measured by DNA fragmentation assays, FACS and Northern as well as Western blot analyses, respectively. **Results:** Stearic acid (C18:0), linoleic acid (C18:2 ω 6), linolenic acid (C18:3 ω 9) and arachidonic acid (C20:4 ω 6) time and concentration dependently triggered apoptosis in HUVECs (n=7, p<0.05) up to 500% of untreated control cells (set as 100%). In contrast, only the highest concentration of oleic acid (C18:1 ω 3) was able to induce apoptosis in HUVECs (+100%) after 48h, but not after 24h. FFAs (n=5, p<0.05) increased the expression of the apoptosis promoter bak (+100%), but reduced that of the apoptosis inhibitor bcl-2 (-25%), thereby shifting bak/bcl-2 to a pro-apoptotic ratio. Exposure of HUVECs (n=6) to polyunsaturated FFAs led to a G₀/G₁ cell cycle arrest probably mediated by increased protein expression of p21^{WAF1/Cip1} (+60%, p<0.01), an inhibitor of cyclin dependent kinases. In addition, FFAs reduced mRNA expression of the atheroprotector clusterin (-40%), of the vasoconstrictor endothelin-1 (-40%) and concentration dependently degraded NF- κ B's inhibitor I κ B (-45%) in HUVECs (n=6, p<0.01). **Conclusions:** FFAs modulate apoptosis, proliferation and the secretory profile of vascular endothelial cells, possibly by activation of the red/ox sensitive transcription factor NF- κ B. By such action FFAs may contribute to the development of endothelial dysfunction preceding late diabetic vascular complications.

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INSULIN INCREASES eNOS EXPRESSION IN HUMAN ENDOTHELIAL CELLS: MODULATION OF THIS EFFECT BY HIGH GLUCOSE.

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Background and Aims: Insulin acutely increases Nitric Oxide (NO) endothelial production inducing the endothelium-dependent vasorelaxation. Hyperglycemia is associated with vascular disease and endothelial dysfunction might be one of the mechanisms for this. However, the molecular pathways of insulin-induced increased endothelial NO production are far from being thoroughly fully elucidated and little is known about the interaction between insulin and elevated glucose concentration in modulating endothelial Nitric Oxide Synthase (eNOS) expression and activity. This study was designed to investigate the effect of insulin on eNOS expression and activity and to determine whether high glucose can modulate this effect.

Material and Methods: Expression of eNOS gene (by semi-quantitative RT-PCR), protein level (by Western Blotting) and production of NO (by conversion of [³H]-L-arginine in [³H]-L-citrulline) were studied in human umbilical endothelial cells (HUVEC) exposed to insulin (10 nmol/L) in presence or absence of high glucose level (25 mmol/L) for 24 hours.

Results: As compared to control cells, in HUVEC cultures exposed to insulin eNOS mRNA levels resulted markedly increased; this effect was substantially reduced by high glucose (2.6 and 1.8 fold increase vs. control, respectively). When control, insulin and insulin plus glucose treated cells were compared, eNOS protein level, as detected by Western blotting, was also greater in cells exposed to insulin alone than in cells exposed to insulin plus glucose. Densitometric analysis showed an almost 2.5 fold increase above control in eNOS protein expression after insulin and 1.9 fold increase after insulin plus glucose addition (OD: 95 \pm 15, 240 \pm 23 and 180 \pm 17 for control, insulin and insulin plus glucose treated cells, respectively, p<0.01). Insulin alone significantly increased NOS activity from 0.1 \pm 0.03 to 0.26 \pm 0.04 pmol/mg protein/min⁻¹ (p<0.05 vs control cells) and this effect was reduced by high glucose (0.17 \pm 0.02 pmol/mg protein/min⁻¹, p<0.05 vs insulin alone).

Conclusions: In human endothelial cells, insulin can increase eNOS gene expression. This effect, as well as insulin stimulation of NOS activity, is impaired in the presence of elevated glucose concentration. Thus our data provide direct evidence that high glucose can impair insulin stimulation of NO synthesis in endothelial cells.

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HIGH FATTY ACIDS PROMOTE CELL GROWTH AND AFFECT CYTOSOLIC CA²⁺ HOMEOSTASIS IN ENDOTHELIAL CELLS

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Background and Aims: Hyperlipidemia is a primary cause of cardiovascular diseases and often associated with diabetes. There is evidence that endothelial cell (EC) dysfunction characterized by diminished endothelium-dependent relaxation occurs upon high levels of fatty acids (FA) in blood, but little is known about the underlying molecular mechanisms. Since nitric oxide (NO) is the major player in endothelium-mediated vascular relaxation and NO synthase in ECs is activated by an increase of cytosolic free Ca²⁺ levels ([Ca²⁺]_i), thus the possible changes in [Ca²⁺]_i were examined. **Materials and Methods:** Bovine aortic endothelial cells (BAECs) were exposed to various levels (0.25, 0.5 or 1 mM) of FA mixture (oleate and palmitate; 2:1) in culture for 2-10 days. Cell growth was assessed by determinations of DNA. [Ca²⁺]_i was measured with a fluorescent Ca²⁺ probe (fura-2). **Results:** Growth of BAECs was increased by 20-27% (P<0.01) after 2-day exposure to 0.25-1 mM FA. Five-day culture with 0.25-0.5 mM FA also enhanced proliferation by 33-47% (P<0.01) whereas 1 mM FA reduced cell growth by 20% (P<0.05). Resting [Ca²⁺]_i was markedly elevated by ~80% (P<0.01) after 5-10 day culture with 1 mM FA. However, the receptor agonist (activating phospholipase C) bradykinin (2 μ M)-evoked [Ca²⁺]_i increments were significantly decreased by ~37% and ~43% (both P<0.01) in BAECs cultured with 1 mM FA for 5 and 10 days, respectively. Furthermore, both bradykinin-induced intracellular Ca²⁺ mobilization and subsequent extracellular Ca²⁺ entry were impaired under these conditions. On the contrary, [Ca²⁺]_i rises evoked by 2 μ M thapsigargin (an agent mobilizing intracellular Ca²⁺ by inhibiting Ca²⁺-ATPase on ER) were not affected by FA culture. **Conclusions:** Exposure to elevated FA promotes EC growth and thus may cause angiogenesis *in vivo*. The defected [Ca²⁺]_i homeostasis by high FA in ECs may reduce NO generation and contribute to the diminished endothelium-dependent relaxation during hyperlipidemia. These FA effects thus increase the risk of developing cardiovascular complications in diabetes.

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Endothelial insulin action and resistance: mechanisms and consequences

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Background and Aims: Resistance to insulin-mediated glucose uptake is associated with endothelial dysfunction and the development of cardiovascular disease, yet the underlying mechanisms remain poorly characterised. We have previously demonstrated that insulin is a direct-acting vasodilator in man, at least in part dependent on nitric oxide (NO) synthesis, and that this vascular action is related to whole-body insulin sensitivity. We have now examined: (i) the molecular mechanisms of insulin action with respect to NO production in cultured human aortic endothelial cells (HAECs); and (ii) insulin-mediated vascular relaxation (IMVR) *ex vivo* in young women with polycystic ovarian syndrome (PCOS), a state of insulin resistance, and no evidence of cardiovascular disease.

Materials and Methods: PI3K recruitment to the insulin receptor and PKB α activity were assessed by immunoblotting of IRS-1/IRS-2 immunoprecipitates and peptide kinase assay of PKB α immunoprecipitates respectively, from HAECs stimulated with insulin. NO production in HAECs was assayed using a Sievers 280A NO meter. Contractile responses to norepinephrine were assessed in resistance arteries <300 μ m internal diameter (from gluteal biopsies) at baseline, after incubation with insulin (100 pM), and after a further incubation with both insulin and L-NMMA (an inhibitor of NO synthase).

Results: Stimulation of HAECs with insulin resulted in rapid, dose dependent recruitment of PI3K to both IRS-1 and IRS-2, with a rapid, sustained, dose-dependent stimulation of PKB α , sensitive to the PI3K inhibitor, wortmannin. In addition, insulin stimulated NO production within 5 minutes. In the resistance artery studies, the ED50 for NE dose-contraction responses was reduced in control vessels by insulin (p<0.05), but not in vessels from patients with PCOS.

Conclusions: In stimulating NO synthase in human endothelial cells, insulin employs a signalling cascade also utilised in upregulating glucose transport in 'conventional' target tissues. The parallel occurrence of metabolic insulin resistance and impaired vascular responses to insulin *ex vivo* in young insulin resistant women in the absence of cardiovascular disease suggests that pathophysiologically relevant insulin resistance occurs in human vascular tissues.

OP 7

Pregnancy: Congenital Malformation and Low Birth Weight

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The Oxidative Stress Indicator 8-iso-PGF2 α is Teratogenic to Rat Embryos In Vitro, and Antioxidants Diminish this Effect.

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Background and Aims: Increased glucose concentration disturbs development in rodent embryos cultured in vitro, and addition of antioxidants diminishes the embryonic dysmorphogenesis. Oxidized arachidonic acid molecules, the isoprostanes, serve as markers of lipid peroxidation and oxidative stress. Increased isoprostane levels are present in embryos subjected to elevated ambient glucose concentration and in offspring of diabetic rodents. Furthermore, the elevated isoprostane concentration in embryos exposed to high glucose levels is decreased by addition of NAC to the culture medium. In addition to their function as indicators of oxidative stress, isoprostanes may have an independent teratogenic role in the dysmorphogenesis of diabetic pregnancy, a notion examined in this study.

Our aim was to investigate if exogenous administration of the isoprostane 8-epi-PGF2 α is teratogenic in vitro, and, if addition of the antioxidants N-acetylcysteine (NAC) or superoxide dismutase (SOD) may modulate such teratogenicity.

Materials and Methods and Results: We subjected rat embryos to 2 μ mol/l of 8-epi-PGF2 α with or without addition of 0.5 mmol/l NAC or 4800 U/ml SOD during 48 hours in vitro culture and compared the outcome with embryos cultured without any of these compounds. We found increased levels of the isoprostane and disturbed development in embryos subjected to the isoprostane (decreased crown-rump length, 3.6 vs. 4.0 mm, decreased somite number, 25 vs. 30, increased malformation score, 5.0 vs. 0.1). Addition of NAC to the isoprostane-exposed cultures normalized the isoprostane content and all morphometric parameters (crown-rump length 4.1 mm, somite number 31, malformation score 0.3), whereas SOD addition had marginal effect (crown-rump length 3.7 mm, somite number 27, malformation score 2.2).

Conclusions: Isoprostanes are teratogenic in vitro, which suggests that these compounds may exert similar effects in vivo. The teratogenic insult appears to involve an excess of radical oxygen species, and is, at least at lower concentrations, diminished by antioxidative treatment.

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Maternal treatment with insulin or antioxidant protects from malformations and DNA damage in offspring of diabetic rats.

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Background and Aims: Previous studies of transgenic BigBlue mice/rats with a lac-I reporter gene have shown increased rates of dysmorphogenesis and DNA damage in embryos exposed to high glucose concentration in vitro, as well as in embryos of diabetic rats. Antioxidative treatment diminishes the embryonic dysmorphogenesis both in vitro and in vivo. These findings support the notion of glucose/diabetes-provoked occurrence of teratogenic oxygen species in these embryos, but do not clarify if the DNA damage is causally related to the embryonic dysmorphogenesis. The aim of the present study was therefore to investigate if treatment of the pregnant diabetic rat with insulin or vitamin E would alter the dysmorphogenesis and rate of DNA damage in the embryos.

Materials and Methods: Normal (N) and diabetic Sprague Dawley female rats were mated with transgenic BigBlue male rats. The female diabetic rats were either untreated (D), or treated with either daily insulin injections (DI), or given 2 % vitamin E in the food (DE). The pregnancies were interrupted on day 11, and the offspring was evaluated with respect to morphology and rate of DNA damage, the latter estimated by the BigBlue rat mutagenesis assay.

Results: Maternal diabetes caused decreased crown-rump length, increased malformation score and DNA mutation rate (N vs. D: 4.01 vs. 3.44 mm, 0.04 vs. 1.85, 1.65 vs. 4.52 $\times 100000$), whereas insulin treatment normalized crown-rump length and DNA mutation frequency (DI: 4.04 mm, 1.88 $\times 100000$), and decreased malformation score (I.11). Vitamin E treatment did not normalize crown-rump length (DE: 3.39 mm), but diminished malformation score and DNA mutation rate (DE: 0.61, 2.85 $\times 100000$).

Conclusions: Maternal diabetes causes increased rate of embryonic dysmorphogenesis and DNA damage. Treatment with either insulin or vitamin E diminishes the developmental disturbances and DNA damage in the embryo.

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Methylglyoxal is less teratogenic than 3-deoxyglucosone due to rapid metabolism in embryos exposed to high glucose concentration in vitro.

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Background and Aims: Embryos exposed to high glucose concentration in vitro show dysmorphogenesis. Production of reactive alpha-oxoaldehydes (methylglyoxal, MG, and 3-deoxyglucosone, 3-DG) with teratogenic capacity may be a consequence of the high ambient glucose concentration. The alpha-oxoaldehydes are potent glyating agents that modify proteins and nucleotides to form advanced glycation endproducts (AGEs). We have previously demonstrated that 3-DG is increased in high glucose-exposed embryos, and that 3-DG has teratogenic capacity. The aim of the present study was to investigate the teratogenicity of MG, and compare it to 3-DG-induced dysmorphogenesis.

Materials and Methods: We subjected day-9 rat embryos to in vitro culture for 48 hr, and supplemented the culture medium with 1 μ M - 1 mM MG, whereupon we assessed the embryonic development. We also measured the activity of the enzymes metabolising MG and 3-DG.

Results: MG induced major embryo malformation only at the highest concentration, 1 mM, (somite number decreased by 5 %, malformation score increased from 0 to 3.6). The embryonic concentration of MG when malformation was induced was ca. 4-5 fold higher than that of embryos suffering hyperglycaemia-induced malformation in vitro. The activity of the MG-metabolising enzyme glyoxalase I in embryos was 2.4-2.7 U/mg protein. Glyoxalase II activity was also present. The 3-DG metabolising enzymes showed much lower activities: 3-DG reductase activity was 170 mU/mg protein, and there was no detectable 3-DG dehydrogenase activity (<0.003 mU/mg protein).

Conclusions: MG is not a critical teratogen in embryopathy induced by hyperglycaemia in vitro, whereas 3-DG is. The major enzymatic pathway for the detoxification of MG, the glyoxalase system, is highly active in the embryos, whereas the 3-DG detoxifying enzymes, 3-DG reductase and 3-DG dehydrogenase, have low and non-detectable activity, respectively. 3-DG may be teratogenic therefore because it is metabolised slowly and accumulates to high levels in the embryo.

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Is there a relationship between major congenital malformations and blood glucose in women with gestational diabetes mellitus?

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Background and Aims: In women with prepregnancy diabetes mellitus, major congenital malformations (CM) are related with periconceptional blood glucose levels and a relationship between fasting blood glucose and CM has also been described in women with gestational diabetes mellitus (GDM). The aim of this work has been to analyze if there is a relationship between CM and the severity of GDM in infants born to mothers with GDM.

Materials and Methods: A universal screening policy and 3rd Workshop-Conference diagnostic criteria have been used for GDM diagnosis. GDM severity has been assessed after prior glucose abnormality, blood glucose values in the diagnostic OGTT, gestational age at diagnosis and requirement of insulin Tx during pregnancy. Potentially confounding variables (age, body mass index and smoking habit) have also been considered. Quantitative variables have been transformed into tertiles to explore their relationship with CM. First, the relationship of each potentially predictive variable with CM has been analyzed with a chi-square test and finally a multivariate logistic regression analysis with CM as the dependent variable has been performed.

Results: 1905 infants born to mothers with GDM have been studied. The rate of CM has been 3.2%. In the bivariate analysis, a positive relationship has been found between CM and prior glucose abnormality (2.8% vs 5.8%, $p < 0.05$), CM and fasting blood glucose (2.3%, 2.6% and 4.7% in each tertile, $p < 0.05$) and CM and BMI (1.6%, 3.3% and 4.7% in each tertile, $p < 0.01$). In the logistic regression analysis, only BMI has remained as predictor of CM (OR 2.39 and 3.41 for the second and third tertiles vs the first one, $p < 0.01$).

Conclusions: In this group of infants born to mothers with GDM, BMI and not the severity of GDM is predictor of CM

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LOW BIRTH WEIGHT IS ASSOCIATED WITH IMPAIRED INSULIN SECRETION AND ACTION IN YOUNG MALE ADULTS

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Background and Aims: Numerous studies have linked low birth weight (LBW) with increased risk of Type 2 diabetes. However, uncertainty still prevails as to the underlying cellular mechanisms.

Materials and Methods: We investigated hepatic and peripheral insulin action (IA), including intracellular glucose metabolism, using the 'gold standard' euglycemic hyperinsulinemic clamp technique at two physiological insulin levels (2x120min; 10mU/m²/min, 40mU/m²/min) in combination with indirect calorimetry and [3-3H]-glucose, in twenty 19-yr old men with LBW (BW 2702±45g) and twenty controls with BW in the upper normal range (BW 3800±22g; p<0.0001), matched for BMI and total fat mass. Insulin secretion (IS) was examined in response to oral (OGTT) and intravenous glucose (IVGTT).

Results: Fasting plasma glucose as well as 2-hour plasma glucose in response to oral glucose was higher in the LBW group (p=0.05; p=0.07). Glycerol at basal and low clamp insulin concentrations was lower in the LBW group (p=0.04; p=0.06), along with non-significantly lower plasma FFA and plasma ketone bodies. Insulin stimulated glycolytic flux was significantly reduced (p=0.03) and suppression of hepatic glucose production during high physiological insulin levels enhanced in the LBW group (p=0.05). Nevertheless, basal and insulin stimulated rates of whole-body total peripheral glucose disposal, glucose oxidation, lipid oxidation, exogenous glucose storage and non-oxidative glucose metabolism was similar in the two groups. Insulin secretion after oral glucose ingestion was lower in the LBW group, as reflected by lower disposition indices expressing insulin secretion in relation to insulin action (DI=IS x IA; 0-30min: p=0.03; 0-120min: p=0.003), whereas insulin secretion in response to iv-glucose was similar in the two groups. Interestingly, 30-min GLP-1 too was lower in the LBW group (p=0.08).

Conclusions: We propose, that reduced insulin stimulated glycolysis precede overt insulin resistance in men with LBW. The observed lower insulin secretion in response to oral but not iv-glucose in LBW men may reflect deficient incretin hormone secretion and/or -action. Finally, lower glycerol levels, i.e. lower rate of lipolysis, could potentially predispose LBW subjects to obesity later in life.

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Relationship between gestational diabetes mellitus and maternal small birth weight.

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Background and Aims: According to the hypothesis of 'thrifty phenotype' the prevalence of diabetes mellitus in adult life is consistently related to a small birth weight. Contrasting results have been however produced about the existence of direct significant relation between presence of gestational diabetes mellitus (GDM) and a small maternal birth weight. This study was designed to answer this question.

Materials and Methods: We studied a group of 515 pregnant women whose birth weight was precisely known. They were then classified as normally tolerant to glucose (n=412) or affected with GDM (n=103) after having been tested by means of a 100-g OGTT performed between the 24th and 28th gestational week. The women were then further categorised according to their birth weight in two further groups: those with birth weight <90th percentile (2,600g; n=59) and those whose birth weight was greater than this cut off value (n=456).

Results: Prevalence of GDM was significantly higher in the group with small birth weight (19/59; 32.2%) as compared to the other group with a normal-high birth weight (84/456; 18.42%; chi-square=6.202; p=0.013). The association between small birth weight and GDM was further reinforced by multiple logistic regression analysis model where age, parity family history of diabetes and pregestational maternal body weight acted as covariates (p=0.028). According to this model the odds ratio of GDM in group of women with low-birth weight was 2.179 (95%CI 1.182-4.016; p=0.012).

Conclusions: According to this study in our population of pregnant women a birth weight <2,600g seems to be significantly associated with a two-fold higher risk for GDM, independently from possible other major confounders.

OP 8 Neuropathy

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The significance of hydroxyl radicals in the aetiology of large and small nerve fibre and neurovascular dysfunction in experimental diabetes

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Background and Aims: Oxidative stress contributes to the development of neural and vascular complications in experimental diabetes. Treatment with broad specificity free radical scavengers such as vitamin E improves nerve conduction and perfusion in streptozotocin-diabetic rats. However, it is not clear which reactive oxygen species are involved or the precise processes important in their formation. To further elucidate this problem, we examined the effects of the specific hydrophilic hydroxyl radical scavenger, dimethylthiourea (DMTU) on measures of large and small nerve fibre function and neural tissue blood flow. **Materials and Methods:** After 6 weeks of streptozotocin-induced diabetes, rats were treated daily for 2 weeks with DMTU (3-100 mg/kg i.p.). Small fibre dysfunction was estimated using behavioural tests for mechanical (Randall-Sellito test) and thermal (plantar test) hyperalgesia. In final experiments, under butabarbital anaesthesia, nerve conduction velocities (NCVs) were measured and sciatic nerve and superior cervical ganglion blood flow was estimated by hydrogen-clearance microelectrode polarography. **Results:** Diabetes caused a 20.8±0.8% reduction (p<0.001) in sciatic motor NCV. This was dose-dependently corrected by DMTU: by 21.0±5.9% (p<0.01) at the lowest dose (3 mg/kg) and by 92.6±3.6% (p<0.001) for 100 mg/kg treatment. The ED50 was approximately 9 mg/kg. Saphenous sensory NCV was also investigated for 100 mg/kg DMTU; a 15.8±0.9% (p<0.001) diabetic deficit was completely corrected (p<0.001). Thresholds for the withdrawal reflex to hindpaw pressure stimulation were 18.9±2.2% (p<0.01) decreased by diabetes, indicating mechanical hyperalgesia, which was 61.9±18.0% (p<0.05) ameliorated by DMTU. Similarly, a 25.0±4.1% (p<0.01) diabetic decrease in the latency for foot withdrawal from a noxious thermal stimulus was 73.1±13.6% (p<0.01) corrected by DMTU treatment. Sciatic nutritive (capillary) and total endoneurial blood flow were reduced by 50.7±3.5% (p<0.001) and 62.5±5.5% (p<0.01), respectively, in diabetic rats. However, with DMTU treatment, these deficits were 86.4±9.7% (p<0.001) and 92.8±26.3% (p<0.01) corrected. There was also a 52.4±3.5% (p<0.001) reduction in superior cervical ganglion perfusion, which was 76.4±9.1% (p<0.001) rectified by DMTU. **Conclusions:** Hydroxyl radical scavenger treatment reverses a wide range of neural and neurovascular defects in diabetic rats. In the context of this experimental model, DMTU treatment had approximately 70 times greater efficacy than vitamin E, which further stresses the importance of hydroxyl radicals for experimental diabetic neuropathy.

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POLY(ADP-RIBOSYL)ATION INHIBITORS CORRECT PERIPHERAL NERVE FUNCTION AND ENERGY METABOLISM IN DIABETIC RATS

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Background and Aims: Poly(ADP-ribosylation) is a response to DNA single-strand breakage caused by hydroxyl and superoxide radicals and peroxynitrite. Activation of poly(ADP-ribose)synthetase (PARS) leads to depletion of its substrate NAD, energy failure, and has been recently implicated in diabetes-associated endothelial dysfunction. Taking into account the important role for oxidative stress and endothelial dysfunction in diabetic neuropathy (DN), we evaluated two specific structurally diverse PARS inhibitors, 3-aminobenzamide (ABA) and 1,5-isoquinolinediol (ISO), on functional and metabolic deficits in the diabetic nerve. **Materials and Methods:** Control (C) and streptozotocin(STZ)-diabetic(D) rats were treated with/without ABA or ISO (30 and 3 mg/kg*d i.p.) for 2 wks after 2 wks without treatment. An intervention approach was used to avoid β-cell regeneration and restoration of normoglycemia which occurs when PARS is inhibited shortly after induction of STZ-diabetes. **Results:** Final blood glucose levels (Mean±SEM) were 366±26, 362±9 and 374±7 mg/dl in D, D+ABA and D+ISO. Both agents were well tolerated and had no side effects. Sciatic motor and digital sensory nerve conduction velocities (m/s) were lower in D (43.7±1.4 and 30.2±0.5 vs 58.1±1.8 and 39.4±1.6 in C, p<0.01 for both) and were completely or partially corrected in D+ABA (56±2 and 36.1±0.6, p<0.01 vs D) or D+ISO (54±1.4 and 35.2±0.5, p<0.01 vs D). Phosphocreatine (PCr) level and PCr/creatinine ratio were decreased in D vs C (p<0.05) and were corrected by both agents. ABA and ISO did not affect any variables in C. PARS activity and NAD levels were similar in the sciatic nerve of C and D. **Conclusions:** Enhanced poly (ADP-ribosylation) is involved in the pathogenesis of DN. PARS inhibitors correct nerve functional and metabolic deficits in D by mechanisms unrelated to restoration of normoglycemia. The findings suggest that *vasa nervorum* rather than neural tissues of PNS is a site of ROS-activated poly(ADP-ribosylation) in short-term diabetes, and are consistent with vascular etiology of, at least, early DN.

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Impaired gastric fundus innervation in experimental diabetes: protection by alpha-lipoic acid treatment.

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Background and Aims: Diabetes causes autonomic neuropathy, multiple organ systems being influenced. The gastrointestinal system is often affected, leading to a variety of symptoms including delayed gastric emptying. This is under the control of non-adrenergic non-cholinergic (NANC) autonomic nerves, for which nitric oxide (NO) is the major neurotransmitter, with vasoactive intestinal polypeptide (VIP) as a co-transmitter. Oxidative stress has been shown to be important in the aetiology of nerve fibre dysfunction in diabetes. Therefore, the aims were to examine the effects of experimental diabetes on gastric fundus NANC neurotransmission and to assess whether the antioxidant, alpha-lipoic acid (LA), could attenuate the development of dysfunction. **Materials and Methods:** Diabetes was induced by streptozotocin (45 mg/kg) in mature male rats. The responses of longitudinal muscle of isolated gastric fundus strips to electrical field stimulation were examined. NANC-mediated relaxation was isolated in preparations precontracted with 5-hydroxytryptamine, in the presence of atropine and guanethidine. Treated diabetic rats were given daily LA (100 mg/kg i.p.) for 8 weeks from diabetes induction. **Results:** Diabetes did not affect the contractile response to 5-hydroxytryptamine. However, the relaxation caused by electrical field stimulation of NANC nerves was progressively attenuated by increasing diabetes duration. Thus, maximal relaxation (16 Hz stimulation) was reduced by $25.9 \pm 3.4\%$ ($p < 0.001$) after 4 weeks and $46.4 \pm 6.7\%$ ($p < 0.001$) at 8 weeks, without any significant further deterioration up to 24 weeks. At the 8 week time point, diabetes caused reduced responses ($p < 0.001$) at all stimulation frequencies examined (0.5 to 16 Hz), deficit being particularly severe (approximately 80%) for low frequencies (0.5-2Hz). LA treatment largely prevented the development of this deficit, giving $82.8 \pm 7.6\%$ protection ($p < 0.001$) for maximum relaxation (16Hz stimulation). Significant improvements ($p < 0.01$) were also apparent at 1 Hz and greater frequencies, although from 0.5-4 Hz, a residual defect remained compared to nondiabetic controls ($p < 0.05$). Experiments with the NO synthase inhibitor, NG-nitro-L-arginine showed that NO-mediated neurotransmission was responsible for 70-80% of NANC relaxation in all groups. There were no significant between-group differences in responses to exogenous VIP. **Conclusions:** The diabetic deficits in gastric fundus NANC responses were largely attenuated by LA, suggesting that this antioxidant may have potential for the treatment of diabetic gastrointestinal autonomic neuropathy.

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The effects of insulin C-peptide on nerve function in diabetic rats are blocked by nitric oxide synthase inhibition.

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Background and Aims: The biological actions of insulin C-peptide are the subject of current interest. C-peptide has been suggested to partially prevent diabetic nerve dysfunction in type 1 experimental models, perhaps by a direct action on neural tissue. Alternatively, C-peptide may have vascular effects including stimulation of the nitric oxide (NO) system. Impaired nerve perfusion contributes to experimental neuropathy, therefore NO-mediated vasodilation might potentially account for any beneficial neural effects of C-peptide. The aim was to examine the C-effects on nerve function and blood flow, and ascertain whether this could be altered by chronic NO synthase blockade. **Materials and Methods:** After 6 weeks of streptozotocin-induced diabetes, rats were treated for 2 weeks with C-peptide (osmotic minipump, 50 pmol/kg/min i.v.) alone or combined with the NO synthase inhibitor NG-nitro-L-arginine (LNNA, 10 mg/kg p.o.). In final experiments, under butabarbital anaesthesia, nerve conduction velocities were measured and sciatic nerve blood flow was estimated by hydrogen clearance microelectrode polarography. **Results:** Diabetes caused $19.8 \pm 0.8\%$ and $15.7 \pm 0.9\%$ reductions ($p < 0.001$) in sciatic motor and saphenous sensory nerve conduction velocity, respectively. C-peptide treatment corrected the motor deficit by $61.7 \pm 2.9\%$ ($p < 0.001$) and the sensory deficit by $77.6 \pm 6.8\%$ ($p < 0.001$); however, values remained somewhat decreased ($p < 0.05$) compared to nondiabetic controls. Cotreatment with LNNA completely abolished ($p < 0.001$) C-peptide effects on both sensory and motor conduction. Sciatic nutritive endoneurial blood flow and vascular conductance were $51.5 \pm 3.6\%$ and $40.8 \pm 3.3\%$ reduced by diabetes ($p < 0.001$), respectively. C-peptide partially corrected these defects (flow $56.9 \pm 7.5\%$, $p < 0.001$; conductance $66.2 \pm 6.8\%$, $p < 0.001$) although a degree of abnormal perfusion remained ($p < 0.05$). Total sciatic endoneurial perfusion was also decreased by diabetes (flow $64.1 \pm 5.3\%$, conductance $55.6 \pm 6.2\%$; $p < 0.01$); a trend for approximately 35% amelioration by C-peptide did not reach statistical significance. LNNA cotreatment markedly ($p < 0.001$) attenuated the nutritive vascular effects of C-peptide, nutritive and total flow / conductance values were within the lower half of the diabetic range. **Conclusions:** C-peptide replacement therapy in the physiological range improves nerve function in experimental diabetes. The magnitude of blood flow elevation and the inhibitory effects of LNNA are entirely compatible with the hypothesis that the neural action of C-peptide is mediated predominantly by a vascular mechanism.

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NEAR-NORMOGLYCAEMIA MAINTAINED OVER 14 YEARS FROM THE DIAGNOSIS OF TYPE 1 DIABETES PREVENTS THE DEVELOPMENT OF POLYNEUROPATHY

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Background and Aims: It has not been previously evaluated whether near-normoglycaemia instituted from the diagnosis of Type 1 diabetes onward may prevent the development of sensorimotor and autonomic neuropathy. To address this point we conducted a long-term prospective study. **Materials and Methods:** We examined 32 newly diagnosed Type 1 diabetic patients (21 male, 11 female) aged 12-36 years who were followed over 14 years. Motor and sensory nerve conduction velocity (MNCV, SNCV), coefficient of R-R interval variation at rest (CV), and clinical assessment were performed at the time of diagnosis and after 3 months as well as after 1, 2, 4, 5, 8, 10, 12, and 14 years. **Results:** During the 14-year period, 10 patients had mean HbA_{1c} levels within the near-normal range of $< 8.5\%$ (mean \pm SEM: $8.1 \pm 0.2\%$; Group 1), whereas 22 patients had mean HbA_{1c} levels $\geq 8.5\%$ ($10.4 \pm 0.5\%$; Group 2). After 14 years, MNCV was significantly faster in Group 1 than in Group 2 in the median (54.7 ± 0.9 vs 51.9 ± 0.7 m/s), ulnar (60.2 ± 1.0 vs 54.0 ± 1.2 m/s), and peroneal nerve (47.6 ± 1.2 vs 42.9 ± 1.0 m/s) and SNCV was faster in the median (57.1 ± 0.6 vs 48.2 ± 1.3 m/s), ulnar (54.7 ± 0.9 vs 48.8 ± 1.4 m/s), and sural nerve (47.5 ± 0.9 vs 42.7 ± 1.0 m/s) (all $p < 0.05$). In Group 1 the average decrease in MNCV and SNCV was -0.11 and -0.17 m/s/year, respectively. These annual slowing rates were comparable to the losses observed over 10 years in a non-diabetic control group showing -0.13 m/s/year for MNCV and -0.15 m/s/year for SNCV, respectively. In contrast, Group 2 showed reductions of -0.36 m/s/year for MNCV and -0.44 m/s/year for SNCV, respectively. In Group 2 there was also a significant decline in CV from $6.0 \pm 0.6\%$ at baseline to $4.0 \pm 0.6\%$ at 14 years as compared with Group 1 in which CV was $6.5 \pm 0.5\%$ at baseline and $6.6 \pm 1.0\%$ at 14 years ($p < 0.05$ for Group 1 vs 2). **Conclusions:** In Type 1 diabetic patients near-normoglycaemia maintained from the diagnosis over the next 14 years 1.) results in a decline in nerve conduction not exceeding the age-related degree of slowing within the physiological range, 2.) prevents an approximately 3-fold faster annual decline in nerve conduction induced by poor glycaemic control, and 3.) prevents the deterioration in cardiac autonomic function.

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Biophysical predictors of foot ulceration, amputation and mortality in diabetes.

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Background and Aims: Foot problems are responsible for an excessive physical, emotional and economic burden in people with diabetes. This study examined various peripheral neurovascular and other general diabetes measures as predictive factors for foot ulceration, amputation and mortality within a six-year follow-up period.

Materials and Methods: One hundred and sixty nine diabetic subjects (without significant peripheral vascular disease) recruited for the study were separated into the following groups: diabetes alone (D, n=51), diabetes with neuropathy (DN, n=67), diabetes with a history of foot ulcers (DU, n=34) and diabetes with Charcot Neuroarthropathy (DCh, n=17) and 22 non-diabetic people comprised the control group. At baseline, all subjects underwent extensive assessment of motor nerve conduction velocity (MNCV), vibration / pressure / temperature perception threshold (VPT/PPT/TPT), peripheral vascular function and other diabetes measures.

Results: Subjects were age / BMI-matched, and diabetes duration was the same for all diabetic groups. The median time to death or study end was 69.5 months. New ulcer development during the outcome period was D = 15.7%, DN = 28.3%, DU = 70.6%, DCh = 70.6% and amputation (at any level) was D = 3.9%, DN = 9.0%, DU = 26.5%, DCh = 11.8%. Mortality occurred as follows: D = 7.8%, DN = 16.4%, DU = 35.3%, DCh = 23.5%. Cox regression was used to determine the relative risk of developing a foot ulcer or having an amputation within six years. Predictors of foot ulceration ($p < 0.05$) were weight, BMI, HbA_{1c}, previous ulcer, autonomic dysfunction, PPT, VPT, MNCV, TPT. Predictors of amputation were previous ulcer, PPT, VPT, MNCV, TPT and calcification. Predictors of mortality were creatinine, previous ulcer, PPT, VPT, MNCV, TPT and calcification. Using multivariate Cox's regression the following independent predictors were found ($p < 0.05$) for ulceration (previous ulcer [MNCV at $p = 0.06$]), for amputation (PPT, calcification) and for mortality (creatinine and MNCV).

Conclusions: Measures of small and large nerve fibre function and calcification, amongst others, can predict ulceration, amputation and mortality in diabetes.

OP 9 Clinical Studies with Insulin Analogues

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Pharmacokinetics of the rapid-acting insulin analog, insulin aspart, in subjects with impaired hepatic or renal function.

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Background and aims: The pharmacokinetics (PK) of insulin aspart (IAsp), a rapid-acting insulin analog, were studied in subjects with various degrees of hepatic impairment (Child-Pugh [C-P] scores 5 to 15, inclusive) and in type 1 diabetes subjects with various degrees of renal dysfunction (creatinine clearance >80 ml/min to <30 ml/min).

Materials and methods: To study the effect of hepatic impairment, 24 male and female subjects (6 healthy normals, 18 with hepatic impairment) ≥ 18 years old, with BMI values ≥ 19 and ≤ 38 kg/m² and fasting plasma glucose values <150 mg/dL, received a single dose (0.06 U/kg IAsp) in an open-label, single-center study. The 18 subjects with hepatic impairment were grouped (6 per group) by C-P scores as having Mild (C-P score 5-6), Moderate (C-P score 7-9), or Severe (C-P score 10-15) hepatic impairment. To study the effect of renal impairment, 18 male and female type 1 subjects (6 healthy, 12 with renal impairment) ≥ 18 years, with BMI values ≥ 19 and ≤ 38 kg/m² received a single dose of IAsp (0.1 U/kg) in an open-label, single-center study. The PK parameters (AUC, C_{max}, T_{max}, CL/F, etc.) were evaluated in all subjects after each was administered a single subcutaneous dose of IAsp. Regression analysis was used to determine whether PK parameters of IAsp were linearly correlated to creatinine clearance for renal impairment or to C-P scores for hepatic impairment.

Results: There were no statistically significant differences in values for PK parameters for IAsp between normal subjects and subjects with hepatic impairment. Additionally, no linear association was found between PK parameter estimates and severity of hepatic impairment as determined by the overall C-P score or by any of the parameters of the C-P score (encephalopathy, ascites, serum bilirubin, serum albumin, prothrombin time). For renal impairment, PK parameters of IAsp were not linearly correlated to creatinine clearance ($r < 0.14$). No safety issues appeared during these studies; IAsp was well tolerated by all subjects.

Conclusions: The results of the hepatic study indicate that PK parameter estimates for IAsp are not significantly different for normal subjects and subjects with various degrees of hepatic impairment. The absorption, distribution, and clearance of IAsp were not affected by renal impairment for subjects not requiring hemodialysis. The safety profile of IAsp should be comparable among diabetes patients with various degrees of hepatic or renal impairment.

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PROMISING RESULTS OF 6 MONTHS TREATMENT WITH INSULIN DETEMIR IN TYPE 1 DIABETIC PATIENTS

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Background and Aims: Insulin detemir is a soluble basal insulin analogue with neutral pH and protracted action. The trial investigated the efficacy and safety of detemir and NPH, administered twice daily (morning and bedtime), in combination with rapid-acting insulin aspart at meals. **Materials and Methods:** This was a 6-month, European, multi-centre, open, asymmetrically randomised (2:1 detemir:NPH), parallel group comparison in Type 1 diabetic patients previously on a basal (once or twice daily) / bolus regimen ≥ 2 months. **Results:** The 447 patients exposed to detemir (301) and NPH (146) had comparable baseline characteristics: age 39.9 ± 13.6 years, BMI 24.6 ± 3.3 kg/m², HbA_{1c} $8.1 \pm 1.1\%$ and diabetes duration 16.8 ± 10.3 years. A total of 425 patients (284 detemir, 141 NPH) completed the trial. After 6 months of treatment, no significant difference was observed in HbA_{1c} or fasting plasma glucose (FPG), between the two groups of exposed patients. In the per-protocol population, i.e. patients who adhered strictly to the treatment regimen described in the protocol, a statistically significant difference in FPG was observed in favour of detemir (-1.47 mM, $p=0.04$). Intra-patient variation in fasting blood glucose (FBG) during the last 7 days of treatment was significantly lower with detemir (SD=3.4mM) compared to NPH (SD=3.8mM), $p<0.001$. Nightly 8-hour plasma glucose profiles (23.00 to 07.00) from a subset of patients (90 detemir, 41 NPH) showed a smooth and stable profile with detemir resulting in lower mean plasma glucose at 07.00 in favour of detemir (7.51 mM versus 9.45 mM), $p=0.013$ with a 95% CI of [-3.47; -0.41]. No statistically significant differences were observed in the incidence of hypoglycaemic episodes or adverse events. **Conclusions:** 6-month treatment with detemir resulted in comparable glycaemic control, but more predictable FBG with lower intra-patient variation compared to NPH. Overall safety profiles were similar between the two treatments.

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MEASUREMENT OF INSULIN DETEMIR AND HUMAN INSULIN IN ADIPOSE AND MUSCLE TISSUE USING OPEN-FLOW MICROPERFUSION.

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Background and Aims: Extensive efforts have been made in order to develop human insulin analogues to cover basal insulin needs. In the case of the soluble basal insulin analogue detemir an intended prolonged insulin action is achieved by means of a low free and biologically active fraction due to the high affinity to albumin. Pharmacokinetics in serum as well as in interstitial fluid influences the pharmacodynamics of insulin and insulin analogues. Thus, the aim of this study was to determine the interstitial concentration of insulin detemir and human soluble insulin (HI) in adipose and muscle tissue in order to investigate the underlying transport mechanism of insulin detemir into its interstitial site of action. **Materials and Methods:** A constant i.v. infusion of insulin (HI at 6 pmol*kg⁻¹*min⁻¹, NN304 at 60 pmol*kg⁻¹*min⁻¹ and 120 pmol*kg⁻¹*min⁻¹) and inulin (reference substance) was given to 10 healthy volunteers in a randomised, open, three-period cross over trial with blood glucose clamped at 5 mmol/L. Open-flow microperfusion (OFM) was used to sample interstitial fluid (ISF) in the subcutaneous adipose tissue of the abdominal region and the medial quadriceps muscle. Corresponding samples of ISF and serum were collected at 80 minutes intervals and analysed for total insulin, insulin detemir (albumin-bound and free fraction) and inulin concentrations. Absolute ISF concentrations were calculated on the basis of the interstitial inulin recovery (inulin in recollectated perfusate / inulin in plasma). **Results:** Constant HI infusion resulted in serum and interstitial adipose/muscle concentrations of (mean \pm SD) 386.0 ± 35.4 pmol/L and $100.3 \pm 54.5 / 129.6 \pm 40.5$ pmol/L (26% / 33% of serum insulin), respectively. The 10-fold higher dose of insulin detemir raised the serum level to 30532 ± 4131 pmol/L whereas total interstitial adipose/muscle concentrations remained comparatively low at $567.6 \pm 200.0 / 792.7 \pm 316.7$ pmol/L (< 3% of serum levels). With the 120 pmol*kg⁻¹*min⁻¹ insulin detemir dose the total serum level reached 61938 ± 10751 pmol/L and interstitial adipose/muscle levels $701.2 \pm 336.2 / 1356.0 \pm 414.9$ pmol/L, respectively (< 3% of serum insulin). **Conclusion:** The relative concentration of insulin detemir in ISF is lower than that of HI. Presumably, this is caused by an impeded transcapillary transport of insulin detemir due to extensive albumin binding.

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BENEFICIAL EFFECTS OF INSULIN GLARGINE COMPARED TO NPH IN SUBJECTS WITH TYPE 1 DIABETES.

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Background and Aims: Insulin glargine (Lantus®), a human insulin analog has constant prolonged action compared to NPH human insulin.

Materials and Methods: In a multicenter, randomized, parallel group study, once a day (QD) insulin glargine was compared to twice a day (BID) NPH in subjects with type 1 diabetes previously treated with multiple daily injections of insulin. A total of 394 subjects (mean age 37.8 years, mean glycohemoglobin (GHb) 7.7%, mean fasting blood glucose [FBG] 9.3 mmol/L) were treated for up to 28 weeks. Subjects received either QD insulin glargine (bedtime) or BID NPH (morning and bedtime) and were allowed preprandial regular insulin as needed.

Results: FBG was lower at endpoint with insulin glargine with an adjusted mean decrease from baseline of -1.38 mmol/L for subjects treated with insulin glargine compared to -0.80 mmol/L for subjects treated with NPH ($p=0.014$). A greater percentage of subjects treated with insulin glargine (32.6%) reached a target FBG of <6.66 mmol/L at endpoint than subjects treated with NPH (21.3%; $p=0.015$). In addition, similar percentages of subjects who received insulin glargine (35.8%) and NPH (35.4%, $p=NS$) achieved GHb $\leq 7\%$ at study endpoint. Following 1 month of titration, the percentage of subjects that reported at least 1 symptomatic hypoglycemia event confirmed by a blood glucose (BG) value of <2.8 mmol/L was less in subjects treated with insulin glargine (73.3%) compared to subjects treated with NPH (81.7%; $p=0.021$). Furthermore, the percentage of subjects reporting at least 1 symptomatic hypoglycemia event confirmed by a BG value <2.0 mmol/L was less with insulin glargine (36.6%) than those treated with NPH (46.2%; $p=0.033$). A low percentage of subjects reported a severe hypoglycemia event confirmed by BG <2.0 mmol/L (insulin glargine 2.6%; NPH 5.1% [$p=NS$]).

Conclusions: QD insulin glargine was more effective than BID NPH in improving fasting glycemic control with fewer subjects reporting symptomatic hypoglycemia events.

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Additional basal insulin during lispro intensive therapy in a randomised multicentre crossover study with a real life design.

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Background and Aims: In an intensive regimen with rapid-acting insulin lispro the waning basal insulinaemia might cause high evening glucose values. This study was performed to evaluate the glycaemic control of patients with type 1 diabetes on insulin lispro intensive therapy with an additional lunchtime dose of NPH insulin.

Materials and Methods: The study was an 10-month randomised multicenter crossover trial. After a 2-month run-in period, subjects injected NPH insulin once (1xNPH) or twice (2xNPH) daily for 4 months in a randomised order. Patients were included if they had HbA1c-levels <8.5%, and had used insulin lispro intensive therapy for >3 months. Efficacy measures were HbA1c levels, 8-point glucose profiles, and hypoglycaemic events. The statistical analysis included a within-patient comparison for crossover trials.

Results: Of 121 randomised patients 104 patients finished the trial. Three out of 17 subjects dropped out because of increased mild hypoglycaemia. The mean HbA1c levels during 1xNPH and 2xNPH were 7.2% (sd=0.93) and 7.1% (sd=0.95), respectively. The within-patient differences in HbA1c levels did not differ significantly (T-test, mean difference=0.06%, p=0.37, 95%CI= -0.073-0.20). The predinner and 2-hours postdinner blood glucose values were lower during 2xNPH, with a mean difference of 0.76 mM (T-test, p=0.004, 95%CI=0.25-1.3) and 0.66 mM (T-test, p=0.027, 95%CI=0.1-1.2), respectively. In the evening hours (18.00-24.00 hours), the frequency of mild and severe hypoglycaemia increased during 2xNPH with a median difference of 0.56 episodes/30 days (range -3.4-5.5, Wilcoxon signed rank test, p=0.001) and of 6.9 episodes/patient year (range -5.8-26, Wilcoxon signed rank test, p=0.007), respectively.

Conclusions: Equal HbA1c levels and increasing frequencies of hypoglycaemia in the evening overshadow the slight improvement of the evening glucose profiles during a regimen with twice daily NPH insulin.

Generalised use of an additional lunchtime injection of NPH insulin cannot be recommended to patients with type 1 diabetes using intensive insulin lispro therapy.

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STRONGER MEAL-RELATED EFFECT OF BIPHASIC INSULIN ASPART 30 COMPARED TO IN-SULIN LISPRO MIX 25 IN TYPE 2 DIABETES

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Background and Aims: Biphasic insulin aspart 30 (BIAsp 30) is a new 'lowmix' formulation consisting of 30% soluble and 70% protamine-crystallised insulin aspart (IAsp). The postprandial glycaemic control obtained with BIAsp 30 was compared with that of biphasic insulin lispro 25 (Mix 25) and that of standard biphasic human insulin 70/30 (BHI 30) under strictly controlled meal test conditions.

Materials and Methods: In this randomised cross-over single-dose trial 61 subjects with type 2 diabetes (60.1±9.4 years; BMI 27.3±3.6 kg/m²; HbA1c 8.3±1.1 %) were exposed on 3 separate study days to the following treatments in random order: BIAsp 30 injected immediately before a standard breakfast, Mix 25 injected immediately before breakfast and BHI 30 injected 15 minutes before breakfast. The dose was 0.40 U/kg for all three treatments. No intermediate- or long-acting insulin or oral anti-diabetic agents were allowed for the 24 hours preceding trial days, and pre-injection blood glucose target levels of 6-10 mM were obtained by night-time SC injection of short-acting insulin if necessary. Mean pre-test fasting serum glucose levels obtained were similar between groups (8.4 - 8.6 mmol/L).

Results: The postprandial serum glucose excursion over 5 hours (EXC0-5h(SG)) was reduced for BIAsp 30 by 10% compared to Mix 25 (16.6±4.4 mmol/l*^h vs. 18.9±6.1 mmol/l*^h; p<0.05) and by 17% compared to BHI 30 (20.1±4.9 mmol/l*^h; p<0.0001). Maximum serum glucose concentration (Cmax) was higher for BHI 30 compared to BIAsp 30 (16.7±2.6 vs. 15.9±2.7 mmol/L, p<0.05) but did not differ between Mix 25 and BIAsp 30 (16.4±3.2. 15.9 vs. 15.9±2.7 mmol/L, NS). Time to maximum serum glucose concentration (tmax) was 13 minutes shorter for BIAsp 30 than for BHI 30 (p<0.01) and 11 minutes shorter than for Mix 25 (p<0.05). These pharmacodynamic results were supported by the pharmacokinetic profiles.

Conclusions: Postprandial glucose was more effectively controlled with the new 'lowmix' formulation BIAsp 30 than with both the conventional BHI 30 and the 'lowmix' formulation with insulin lispro, Mix 25. Consequently, BIAsp 30 may be the preferable choice of the three enabling successful treatment in insulin-resistant Type 2 diabetic patients.

OP 10

Health Care Delivery, Education and Economics

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HbA1c and severe Hypoglycaemia after intensified treatment and education of 10000 type 1 diabetic patients. Results of a ten years nationwide quality-circle.

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Background and Aims: Development and implementation of a nationwide continuous quality management for intensified insulin therapy in clinical routine.

Materials and Methods: A peer-review quality circle was formed as an official working group of the GDA based on the formation of a working group (ASD) of presently 139 general internal medicine departments from city, country and university hospitals. The structured treatment and teaching programme for ICT includes multiple daily insulin injections (ICT) or continuous subcutaneous insulin infusion (CSII), several times daily BG self-monitoring, self-adaptation of insulin dosages by the patients themselves and a far-reaching liberalization of nutrition. The group attempted to document and to improve the quality of structure and process of type 1 diabetes care in its participating institutions by a system of peer supervision. Systematic follow-up examinations of fifty consecutive type 1 diabetic patients 12-15 months after participation in the programme confirm the outcome quality. The PC-System (DIQUAL) was developed for collecting, checking and pooling of the outcome data.

Results: From 1992 up to 2001 a representative sample of 10912 patients with type 1 diabetes was examined. HbA1c (mean normal 5.0%) improved from 8.1 to 7.15%. The incidence of severe hypoglycaemia fell from 0.355 to 0.164, ketoacidosis with hospitalisation from 0.093 to 0.030 and the hospitalisation (all causes) from 5.89 to 3.76 days/patient/year. In the 2001 evaluation patients with a baseline HbA1c >=8% (37%) had an improvement from 9.8% to 8.25% together with a reduction of severe hypoglycaemia from 0.21 to 0.13/patient/year, whereas patients with an acceptable initial HbA1c (<8%; 63%) severe hypoglycaemia could be reduced from 0.37 to 0.13/patient/year with only slight increase in HbA1c from 6.65 to 6.75%. HbA1c (baseline to reexamination) of the patients with CSII (16%) was not better than with pen therapy (8.0 to 7.65% vs. 7.85 to 7.3%), but the reduction in severe hypoglycaemia was marked (CSII 0.37 to 0.10; ICT 0.30 to 0.13). Ketoacidosis was higher in CSII (0.11 to 0.15) than in ICT (0.08 to 0.04).

Conclusions: Participating institutions reached a metabolic control comparable to the DCCT but with less hypoglycaemia. Caring for patients with insulin pump therapy should be restricted to very experienced institutions.

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THE BURDEN OF TYPE 2 DIABETES COMPARED TO THE BURDEN OF TYPE 1 DIABETES IN PATIENTS UNDERGOING INTENSIVE AND SPECIALIZED CARE.

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Background and Aims: Continuous development of the quality of diabetes care is essential to monitor the progress towards the reduction of all complications of diabetes. We have assessed indicators of diabetes care quality in a specialized ambulatory care unit where intensive management of both DM-1 and DM-2 have been implemented since 1992. **Materials and Methods:** All Type 1 and 2 diabetic patients (1,100) attending the annual evaluation (year 1997) were invited to participate. Descriptive analysis of 114 indicators was performed (DC Q-Net, EU Consortium protocol, OptiDiab System). The quality of the processed data were monitored by detection of incompleteness and discordance between electronically captured data and the information shown in patients medical records. SPSS for Windows (v 8.0) was used for statistics, expressing qualitative variables in percentage, and quantitative estimations as mean or median (sd). Chi-square, Student T and Mann-Whitney tests were used for the comparison of results between DM-1 and DM-2 patients. **Results:** 797 patients (442 DM1, 355 DM2). Mean age (y): 39(15) versus 65(11); known diabetes duration, 15(11) vs 16(10). Significant differences were depicted for the prevalence of obesity (females: 0.28,0.67; males: 0.13, 0.41), tabaquism (0.43 vs 0.19); legal blindness (LB) (0.02, 0.07), CHD (0.01,0.10), CVD (0.01,0.06), ESRD (0.01,0.04), foot amputations (FA) (0.01,0.04) incidence of CHD (0.02,0.11), claudication of lower extremities (0.03,0.17) and peripheral polineuropathy (PNP) (0.14,0.32), prevalence of cataracts (0.05,0.25), retinopathy (DR) (0.29,0.43), macular edema (ME) (0.05,0.10), advanced ocular disease (AOD) (0.04,0.14), decreased paresthesia (0.21,0.47), lack of sensitivity to monofilament (0.14,0.33), absence of pedal pulses (0.08,0.18), hypertension (HBP) (0.14, 0.34), macroalbuminuria (MA) (0.08, 0.24), hypertriglyceridemia (HTG) (0.04, 0.21), reduced HDL-c (females: 0.13, 0.48; males: 0.05, 0.21). Mean HbA1c values were similar in both groups: 8.07(1.67), 8.26(2.03).

Conclusions: The rate of complications was much greater in the DM-2 group (20x for ME, 10x for CHD, 6x for CVD, 5.25x for HTG, 5x for cataracts, 4x for ESRD and FA, 3.5x for AOD and LB, 3.0x for MA, 2.43x for HBP and more than 2x for PNP and PNP). Incidence of new CHD episodes (5.5x), and PVD (5.7x) were also greater in type 2 diabetic patients. The study illustrates the need for an anti-smoking campaign, particularly among the youngest patients.

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TRUE OUTCOMES FIVE YEARS AFTER THE IMPLEMENTATION OF QUALITY MANAGEMENT OF DIABETES CARE IN AUSTRIA

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Background and Aims: Open benchmarking and the implementation of quality circles was considered being a feasible approach for improved management of diabetes care. A crucial element in this quality management approach was an effective and highly frequent reporting mechanism. **Materials and Methods:** The group initiated quality circles for the purpose of diabetes case discussions. Quality circles met at least four times per year, a national quality circle had two meetings per year. Quarterly reports were sent to all participants via e-mail or ground mail, dependent on their technical infrastructure. A peer group of 35 GPs and Hospitals was provided with a benchmarking software for diabetes care. Biannual reviews of understanding the acuteness of items for data collection were performed within the national quality circles. The data were additionally analysed according to the diabetes aggregated dataset of the World Health Organisation (WHO). The results were aggregated and transferred to the datawarehouse of WHO annually. Results: 11000 patient examinations in Austria (1996-2000, 100 centers) have been registered. The results show Austrian data between 1996 and 2000 about true outcomes (incidence) of diabetes care.

	Pregnancy Compl	Amputations	Blindness	Renal Failure	Stroke	Myocardial Infarction
1996	n.a.	n.a.	0,53%	1,07%	0,53%	2,14%
1997	0,15%	n.a.	0,33%	0,83%	1,81%	3,45%
1998	n.a.	0,23%	0,14%	0,54%	1,63%	1,45%
1999	0,18%	0,07%	0,17%	0,22%	1,61%	1,81%
2000	0,08%	0,15%	0,08%	0,38%	1,84%	1,83%

Table 1: St. Vincent true outcome incidences (%) in diabetic patients in the Austria area (n.a.=insufficient data). **Conclusions:** Open benchmarking leads to controlled processes. Controlled processes lead to improved outcomes of diabetes care. Substantial improvement could be achieved at newly diagnosed amputations, blindness and renal failure. It has to be stated that these results were obtained in a limited area with a certain numbers of participants, which does not reflect nonpopulation based situation.

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METABOLIC OUTCOME ACHIEVED BY INTENSIVE MANAGEMENT IN EUROPEAN DIABETES CENTERS ARE FAR FROM DESIRABLE TARGETS.

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Background and Aims: Prospective studies (DCCT, UKPDS) have shown the benefits of intensive management (IM) on DM-1 and DM-2. European-wide information regarding long-term metabolic outcome of IM in daily practice is scant and controversial. We report the values of main metabolic indicators for DM-1 and DM-2, achieved after 5 years of IM implementation in the Diabetes Center, and their comparison with National and European networks.

Materials and Methods: All subjects classified as DM-1 and DM-2 (clinical parameters, basal and post-glucagon C-peptide, ICA, GAD, IA2 at diagnosis) were invited to the study, at the time of the annual evaluation in the year 1997 (written informed consent, approval by the Hospital Ethics Committee and the National Data Protection Agency). Most DM-2 patients had been referred after unsuccessful surveillance at primary care level. A total of 797 patients (442 DM-1, 355 DM-2) participated. OptDiab is a computerized diabetes management system designed, ad hoc, after the experience of previous enrolment as a partner in various EU Health Care-Telemedicine Consortia. Local information was aggregated, anonymized and compared with that of 13 national collaborative institutions; national data were equally processed with institutions from other four European networks (DC-QNet). Statistical analysis used SPSS (v. 8.0), expressing data in numbers (mean, sd), and percentages; for comparisons, chi-square and Fischer exact tests were used; significance* implied p<0.05.

Results: A structured education plan was followed by 90.7% of all patients. SMCBG was observed by 89.6% of DM-1 subjects versus 81.6% of DM-2 patients. More than 78 % of DM-1 patients received at least 3 daily injections of insulin or sc pump therapy. In the DM-2 population, 12% were not pharmacologically treated, 18.6% received oral agents, and 73.4% received various insulin regimens, from combined therapy to IIT. Acute metabolic episodes (severe hypoglycemia, hyperglycemia/ketosis) occurred in 25.0% vs 13.5%*. Mean HbA1c was 8.07(1.67) vs 8.26(2.03) (<6.5%:12.2%, 12.9%; <7.5% : 28.6, 30.1%; >7.5%: 59.2%, 57.0%. Aggregated HbA1c levels (DCCT adjusted) recorded at the annual evaluation (1997) from 5 EU countries (DC-QNet, more than 22,000 patients) were 26.9% optimal, 23.2% acceptable and 49.9% poor.

Conclusions: Long term metabolic outcome of patients under IM in European specialized centers are far from achieving the desired goal (HbA1c<m+ 4sd, non-diabetic population).

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LONGITUDINAL EVALUATION OF THE OUTCOME INDICATOR MICRO-ALBUMINURIA BASED ON DATA OF THE DIAB CARE FAX SYSTEM

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Aims: Is a quality management programme (DIAB CARE Fax System) able to develop quality in diabetes care? **Methods:** Structured documentation of 543 patients were obtained 1998 and 1999. The participating doctors had the possibility of continuous data evaluation in benchmarking graphs. Vascular risk factors (microalbuminuria (MAU), blood pressure (Bp) ≥ 130 or ≥ 80 mmHg, HbA1c $\geq 7.5\%$) were monitored. **Results:** In a cohort of 184 patients (type1/type2: n=57/126) MAU was present. From these patients subsequent analysis were performed. The duration of diabetes was (min/median/max): type1: 2/11/35, type2: 2/10/33, age: type1: 10/34/75, type2: 37/64/94, gender (m/f): type1: 28/29, type2: 65/61. 75.4% (type1) and 82.5% (type 2) patients remained in the risk profile of MAU, however 18.6% (type1) and 28.8% (type2) improved their MAU values till 1999. Additionally these 184 patients were analysed after combination of high risk factors. Data are mean values \pm SD.

	MAU + blood pressure ≥ 130 or ≥ 80 mmHg		MAU + HbA1c $\geq 7.5\%$	
	Type 1 (n)	Type 2 (n)	Type 1 (n)	Type 2 (n)
1998	135.8 \pm 17.1/83.2 \pm 8.2 (27)	149.4 \pm 18.1/86.4 \pm 11.7 (81)	9.3 \pm 2.3 (17)	9.1 \pm 1.1 (66)
1999	133.3 \pm 23.1/84.0 \pm 13.1 (19)	137.8 \pm 18.2/83.4 \pm 11.4 (71)	7.7 \pm 1.6 (9)	7.4 \pm 1.1 (27)

83 patients with MAU and HbA1c at risk were evaluated. 58.8% (type 1) and 40.9% (type 2) remained in the high risk level; but in 60% (type 1) and 70.4% (type 2) their follow up HbA1c values improved. A decrease of HbA1c at risk could be shown in 76% (type 1) with insulin and 87.9% (type 2) with treatment of OAD and/or insulin. 44.4% (type1), 89.6% (type 2) of 108 patients with MAU and Bp at risk remained till 1999 in the risk profile. However, the analysis showed that 44.4% (type1), 59.3% (type2) obtained an antihypertensive therapy. 66.7% (type 1) and 45.8% (type2) of them improved their Bp values at risk. **Conclusion:** DIAB CARE BAVARIA uncovers deficits in diabetes care by evaluations based on guidelines. The results reveals the difficulties in translation of activities in quality development in daily practice. However DIAB CARE provides the instruments to support quality circles with analysis, feedback evaluations and expert advice.

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The in-patient costs in the Chinese patients with diabetes from 1995-1999

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Objective To investigate the in-patient cost change in diabetic patients from 1995-1999. **Method** According to ICD-9 code and the standard forms for the investigation of medical expenditure, all the patients with diabetes were analyzed for their hospital costs. **Results** 948 cases (1509 admissions) with diabetes were used for the analysis. The average total cost was 2383 RMB (1US\$=8.3RMB), including 950 for the drugs, 621 for the examinations, 524 for the bedding and nursing in 1995, and was increased annually to total 4850 RMB, 1734 for the drugs, 1198 for the examinations, 1564 for the bedding and nursing in 1999 respectively, which means 103.7%, 82.5%, 92.9%, 198.5% increased. The average time in hospital was decreased from 24.6 in 1995 to 22.6 days in 1999. These diabetic patients with cerebral infarction, with hypertension, with cholecystitis and/or gallstones, with upper respiratory infection, had spent 2.02, 1.89, 1.32, 3.70 times more money than their counterparts without diabetes(4390 vs. 2175, 3560 vs. 1887, 4103 vs. 3102, 2285 vs. 618 RMB, p<0.01). **Conclusion** The in-patient medical costs for diabetic patients dramatically increased last 5 years. The diabetic patients admitted for other diseases spent much more money than their counterparts without diabetes. The health economics in diabetes should be paid more attention, particularly in China which has biggest diabetic population and is fast developing country.

OP 11

Incretin Hormones

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CD26 KNOCK-OUT MICE HAVE IMPROVED GLUCOSE TOLERANCE AND ELEVATED LEVELS OF INTACT INCRETIN HORMONES.

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Background and aims: Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic hormone (GIP) are incretin hormones that enhance glucose-dependent insulin secretion. Both hormones are degraded by dipeptidyl peptidase IV (CD26) forming inactive truncated metabolites. Homozygous CD26^{-/-} knock-out mice lack CD26 enzyme. The aim of this study was to investigate the influence of both incretin hormones on insulin secretion and glucose tolerance in CD26^{-/-} and wild-type mice. **Materials and methods:** 36 male C57BL/6 wild-type and 36 CD26^{-/-} mice were used. Oral glucose tolerance tests (OGTT, 2g/kg) were carried out in 10 week old mice fasted for 18 hours. Tail blood was collected at -30, 0, 30, 60 and 120 min, and blood glucose concentrations were measured. Four weeks later a similar glucose challenge was given, and orbital blood was collected 15 min after for plasma hormone determinations. GLP-1 and GIP were measured by specific RIAs directed towards the C-terminal (intact + truncated peptides) and N-terminal (intact, biologically active peptides) regions of each hormone. **Results:** The area under the glucose curve during OGTT was reduced ($p < 0.01$) in the CD26^{-/-} mice compared to wild-type (550 ± 32 and 766 ± 60 mM \cdot min). Insulin levels were increased ($p < 0.0001$) in the CD26^{-/-} mice, 232 ± 16 vs 135 ± 12 pM in wild-type mice. Furthermore, concentrations of intact, biologically active GLP-1 and GIP were elevated in the CD26^{-/-} mice (GLP-1, 9.8 ± 1.0 vs 6.1 ± 0.8 pM, $p < 0.05$ and GIP, 110 ± 10 vs 31 ± 2 pM; $p < 0.0001$). However, there was no significant difference in the total amount of incretin hormones secreted (total GLP-1, 8.4 ± 0.7 vs 7.8 ± 0.3 pM, $p = 0.38$, and total GIP 129 ± 17 vs 175 ± 19 pM, $p = 0.09$, CD26^{-/-} vs wild-type). **Conclusion:** These results demonstrate that lack of CD26 enzyme activity results in elevated concentrations of the intact, biologically active forms of GLP-1 and GIP, explaining the increased insulin secretion and improved glucose tolerance in the CD26^{-/-} mice.

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GLP-1 ACTIVATES PI3K AND PKB IN RAT SKELETAL MUSCLE

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Background and Aims: GLP-1 exerts, in rat and human myocytes, insulinomimetic effects upon glucose metabolism, which are not mediated by activation of PKA, and that are associated to an increased production of an inositolphosphoglycan, considered as a second messenger for insulin action. In this work, we have explored whether GLP-1 activates PI3K and PKB, and if its glycogen synthase activation effect is affected by wortmannin, a potent PI3K inhibitor. **Materials and Methods:** Strips of the pair soleus muscle (two strips per muscle) from one Wistar rat were used for each experiment. Glycogen synthase α activity (GSA) was measured as the incorporation of D-[U-¹⁴C] glucose (0.75 μ Ci) into glycogen, in the presence of 5 mM D-glucose, in muscle samples preincubated for 60 min at 37°C in KRB with 1% BSA, and then incubated for 10 min in the absence (control) and presence of 10^{-10} M GLP-1 or 10^{-9} M insulin, and all in the absence and presence of 10^{-6} M wortmannin. PI3K activity -measured as the PIP3 formation-, and phosphorylation of PKB -by Western blotting-, was assayed in muscle strips treated in the same conditions as for GSA, but incubated during 3 min. Data are presented as mean \pm SEM, and significance were assessed by Student's *t*-test. **Results:** 10^{-10} M GLP-1 exerted a significant stimulation in GSA activity [$173 \pm 13\%$ of control (3.1 ± 0.2 U/g, $n = 8$), $n = 3$, $p < 0.05$], which was apparently higher than that induced by 10^{-9} M insulin ($134 \pm 2\%$ of control, $n = 5$, $p < 0.001$); the additional presence of 10^{-6} M wortmannin completely abolished the increment induced by either GLP-1 ($98 \pm 32\%$ of control, $n = 3$) or insulin ($89 \pm 9\%$ of control, $n = 3$). Also, GLP-1 stimulated both, the PIP3 formation ($126 \pm 8\%$, $n = 3$, $p < 0.02$) and the phosphorylation of PKB ($222 \pm 25\%$, $n = 3$, $p < 0.001$). **Conclusions:** In rat skeletal muscle, GLP-1 activates PI3K and PKB, and when incubated in the presence of wortmannin, the GLP-1 induced increment in glycogen synthase α activity was abolished, indicating that PI3K, and probably PKB, are implicated in the activation of the enzyme by GLP-1.

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Inhibition of GIP/GIPR axis prevents obesity

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Background and Aims: Secretion of gastric inhibitory polypeptide (GIP), which is a potent insulinotropic peptide released from intestinal K cells after absorption of glucose or fat, is elevated under a high fat diet. In this study, we examined the physiological role of elevated GIP secretion on diet-induced obesity and glucose homeostasis.

Materials and Methods: Mice with GIP receptor (GIPR) deficiency were used in following experiments. Wild type (GIPR^{+/+}) and GIP receptor deficient (GIPR^{-/-}) mice were fed a high fat (HF) diet from 7 weeks of age to 50 weeks.

Results: While GIPR^{+/+} mice showed 35% body weight gain under HF diet (control 28.3 ± 0.5 g vs. high fat 41.3 ± 1.9 g, $P < 0.01$), accompanied with the increased visceral and subcutaneous fat mass, fatty liver and adipocyte hypertrophy, GIPR^{-/-} mice did not show HF diet induced obesity (control 29.5 ± 1.7 g vs. high fat 31.0 ± 1.1 g). In oral glucose tolerance test, HF diet fed GIPR^{+/+} mice showed hyperinsulinemia (control 2204 ± 239 pg/ml vs. HF 5214 ± 586 pg/ml at 30 min, $P < 0.05$) with elevated fasting blood glucose levels. On the other hand, although HF diet fed GIPR^{-/-} mice had mild glucose intolerance compared to GIPR^{+/+} mice, GIPR^{-/-} mice did not show hyperinsulinemia (control 1664 ± 23 pg/ml vs. high fat 2041 ± 161 pg/ml). In insulin tolerance test, although GIPR^{+/+} mice fed HF diet revealed obvious insulin resistance, GIPR^{-/-} mice fed HF diet are as insulin sensitive as GIPR^{-/-} mice fed a control diet. However, no significant differences were observed in the food intake or rectal temperature in either group of GIPR^{+/+} and GIPR^{-/-} mice.

Conclusions: These results propose that elevated GIP secretion has pivotal role in diet-induced obesity, indicating that GIP acts as the thrifty gene.

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Glucagon-Like Peptide-1 for 6 Weeks Improves Glycemic Control, Insulin Sensitivity and β -cell Function in Type 2 Diabetic Patients

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Background and Aims: The intestinally produced peptide hormone Glucagon-like Peptide-1 (GLP-1) has been shown to reduce plasma glucose in type 2 diabetic patients. Long-term effects of the hormone have never been examined. We therefore examined the effects of 6 weeks of GLP-1 infusion on glycemic control, insulin sensitivity and β -cell function in type 2 diabetic patients.

Materials and Methods: 20 patients with type 2 diabetes were randomly allocated to receive continuous s.c. infusion of GLP-1 or saline. Infusion rate: 4.8 pmol/kg/min. The patients were matched for sex, age, BMI and fasting plasma glucose. Before (week 0), after 1 week (week 1) and after 6 weeks (week 6) of infusion β -cell function (hyperglycemic clamp at 30 mmol/l) and 8-h profiles of plasma glucose, hormones and FFA were measured. Before (week 0) and after 1 week (week 1) of infusion, HbA1c, fructosamine and insulin sensitivity (hyperinsulinemic euglycemic clamp) were measured.

Results: For patients receiving saline all measurements remained unaltered during the 6 weeks. For patients receiving GLP-1 fasting plasma glucose decreased from 14.4 ± 1.0 (week 0) to 10.8 ± 0.9 (week 1) to 10.1 ± 1.1 (week 6) mmol/l, $P < 0.0001$. 8-h mean plasma glucose decreased from 15.8 ± 1.2 (week 0) to 10.9 ± 1.0 (week 1) to 10.3 (week 6) mmol/l, $P < 0.0001$. Fasting levels of FFA decreased from 0.9 ± 0.08 (week 0) to 0.77 ± 0.09 (week 1) to 0.63 (week 6) mmol/l, $P = 0.0005$ (week 0 vs week 6). 8-h mean levels of FFA decreased from 0.64 ± 0.08 (week 0) to 0.55 ± 0.06 (week 1) to 0.49 ± 0.06 (week 6) mmol/l, $P = 0.01$ (week 0 vs week 6). HbA1c decreased from 9.2 ± 0.6 (week 0) to 7.3 (week 6) %, $P = 0.003$. Fructosamine decreased from 349 ± 23 (week 0) to 282 ± 16 (week 6) μ mol/l, $P = 0.0002$. Insulin sensitivity increased from 2.1 ± 0.7 (week 0) to 3.9 ± 1.0 (week 6) mg glucose/kg lean body mass/min, $P = 0.003$. First phase responses of the β -cell (incremental AUC levels of C-peptide 0-10 min) increased from -249 ± 509 (week 0) to 1936 ± 1124 (week 1) to 3400 ± 919 (week 6) pmol \cdot min, $P = 0.02$. Maximal secretory capacity (C-peptide) increased from 2618 ± 383 (week 0) to 5040 ± 836 (week 1) to 5690 ± 893 (week 6) pmol/l, $P < 0.0001$.

Conclusions: In type 2 diabetic patients continuous subcutaneous infusion of GLP-1 reduces fasting and 8-h mean plasma glucose by 4.3 and 5.5 mmol/l. HbA1c decreases by 1.3% and fructosamine normalises. Fasting and 8-h mean levels of FFA decreases by 30 and 23 % respectively and insulin sensitivity improves by 84%. Furthermore β -cell function improves.

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THE GLP-1 ANALOGUE, NN2211, INHIBITS FREE FATTY ACID-INDUCED APOPTOSIS IN PRIMARY RAT β -CELLS.

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Background and aims: NN2211 is a long-acting GLP-1 derivative with pharmacokinetic properties suitable for once daily administration in humans. It has been demonstrated both in vivo and in vitro that that GLP-1 can increase the β -cell mass. It has not been investigated whether this could be due to inhibition of β -cell apoptosis. **Materials and methods:** Pancreatic islets were isolated from neonatal rats. Islets were incubated for 16 hours with 1 mM free fatty acids (2:1 oleic acid:palmitic acid) with or without NN2211 or GLP-1. Islets were dispersed, stained with the DNA binding dye 7-AAD and submitted to flow cytometric analyses of DNA content. In separate experiments, apoptosis was induced in the β -cell lines RIN and INS-1 as above and cellular viability was assessed using a standard MTT assay. **Results:** Approx. 60% of the primary β -cell displayed fragmented DNA as a sign of apoptosis when incubated with free fatty acid for 16 hours. NN2211 reduced the frequency of apoptotic cells in a dose-dependent manner, reaching 50% inhibition of apoptosis at 100 nM ($p < 0.005$). GLP-1 also protected β -cell from free fatty acid-induced apoptosis but only by 25 % at 100 nM ($p < 0.01$). Thus, NN2211 was 2-fold more efficient than native GLP-1 in protecting β -cells from free fatty acid induced apoptosis ($p < 0.01$). The anti-apoptotic effect of NN2211 was GLP-1 receptor specific as NN2211 protected GLP-1 receptor positive but not GLP-1 receptor negative β -cell lines from free fatty acid induced apoptosis. The anti-apoptotic capacity of NN2211 could be mimicked by addition of the cAMP-inducing agent forskolin. In addition, the anti-apoptotic effect of NN2211 could be partially blocked by the PI3 kinase inhibitor wortmannin ($p < 0.005$), but not by the protein kinase A inhibitor H98. **Conclusions:** These data demonstrate that GLP-1 and the long-acting derivative, NN2211, are potent inhibitors of free fatty acid-induced apoptosis in primary β -cells and that the anti-apoptotic signaling of these hormones are dependent on pathways involving cAMP and the PI3 kinase but not the protein kinase A.

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Normal Early Phase but Defective Late Phase Insulin Secretion in Type 2 Diabetic Patients in response to GIP

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Background and Aims: The incretin hormone glucagon-like-peptide (GLP)-1 is strongly insulinotropic in type 2 diabetic patients, whereas glucose-dependent insulinotropic polypeptide (GIP) has little or no effect in these patients. In the present investigation we evaluated "early and late phase" insulin and C-peptide secretion in response to GLP-1 and GIP stimulation in patients with type 2 diabetes mellitus.

Materials and Methods: Eight type 2 diabetic patients (age: 55(49-59) years; BMI: 29.5(27.6-34.4) kg/m²; FPG: 10.1(7.6-13.7) mmol/l, HbA1C: 7.4(5.3-9.8)%) were studied on 4 different experimental days. For comparison 6 matched healthy subjects were examined. During a hyperglycaemic clamp (15 mmol/l) we infused (per kg body weight/min) either: 1 pmol GLP-1 (7-36)amide, 4 pmol GIP, 16 pmol GIP (n=4) or no incretin hormone (n=5).

Results: In type 2 diabetic patients total early phase (0-20 min) insulin (mean \pm SEM) and C-peptide (in brackets) AUCs(0-20min) were: 3.55 \pm 0.73 (26.62 \pm 2.37) 20min x nmol/l during GLP-1 infusion, and 3.06 \pm 0.93 (24.06 \pm 3.61) nmol x min/l during low dose GIP infusion (NS). Corresponding results were: 2.39 \pm 0.59 (22.08 \pm 1.81) after glucose only. The "late phase" (20-120 minutes) insulin and C-peptide responses (AUC) were: 97.21 \pm 41.66 (374.94 \pm 63.67) 100min x nmol/l after GLP-1 stimulation and 22.19 \pm 7.61 (186.64 \pm 35.60) 100min x nmol/l after GIP (4 pmol) in type 2 diabetic patients ($p < 0.01$ ($p < 0.01$)). Corresponding responses were 16.14 \pm 5.19 (146.04 \pm 24.74) after glucose only. "Late phase" insulin secretion in response to GLP-1 stimulation in type 2 diabetic patients was indistinguishable to the response to glucose alone in the healthy subjects. With GIP (low + high dose) "late phase" insulin secretion was similar to glucose alone in type 2 diabetic patients. Total amount of glucose (g) given after 2 hours (mean \pm SEM): GLP-1: 61.5 \pm 8.5 g; GIP: 33 \pm 3.6 g and no incretin hormone: 29.8 \pm 5.3 g.

Conclusions: We conclude that type 2 diabetic patients have preserved "early and late phase" insulin and C-peptide responses to GLP-1, whereas they have a defective late phase response to GIP, which may contribute to the pathogenesis of type 2 diabetes mellitus.

OP 12

Signalling in Beta-Cells

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Abnormal responsiveness of insulin release to exogenous pyruvate in patients with physical exercise-induced hyperinsulinemic hypoglycemia

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Aims: We have recently described two patients, in whom strenuous physical exercise leads to hypoglycemia caused by inappropriate insulin release (exercise-induced hyperinsulinism; EIHI). In the normal pancreatic beta cell, exogenous pyruvate does not stimulate insulin release. We hypothesized that the increased levels of lactate and/or pyruvate during anaerobic exercise would trigger the aberrant insulin secretion in patients with EIHI. The aim of this study was to test this hypothesis.

Methods: Ten patients (6 females and 4 males from 3 families) were diagnosed to suffer from EIHI, based on hypoglycemia and more than 3-fold increase in plasma insulin induced by a 10 min bicycle exercise test. Sodium pyruvate (8.2 mmol/m²) was infused intravenously in 60s, and plasma insulin levels were measured at 1, 3, 5, 10 and 30 min. Six healthy controls were tested in a similar manner.

Results: Blood pyruvate level peaked 6.5-fold (from 49 to 307 μ mol/l) in the patients but only 3.2-fold in the controls ($p < 0.05$). Blood glucose levels were significantly lower at 10 min in the patients than in the controls (3.8 vs. 4.7 mmol/l, $p < 0.05$). Insulin secretion in the healthy controls did not respond to the pyruvate bolus. However, all EIHI patients responded to pyruvate with a brisk increase in their plasma insulin (1+3 min response 79 \pm 31 mU in the patients, vs 6 \pm 0.7 mU in the controls, $p < 0.05$). The mean stimulation index was 5.1-fold in the patients and 0.9-fold in the controls ($p < 0.01$).

Conclusions: Our findings indicate that EIHI is an autosomally dominantly inherited trait characterized by abnormal pyruvate-induced insulin release. Its pathogenesis may involve monocarboxylate transport or metabolism in the beta cell. EIHI is a new hyperinsulinemia syndrome which may be more common than has been realized. The pyruvate test provides a simple, safe and specific diagnostic test for this condition.

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Characterization of the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase expression in pancreatic beta cells

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Background and Aims: Glucokinase (GK) plays a key role in the process of glucose recognition in pancreatic beta cells and of glucose metabolism in liver. A phase display library screening revealed the bifunctional glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2) as a GK binding protein. In two-hybrid studies the GK interaction with the PFK-2 liver isoform was shown. It was the aim of this study to elucidate which PFK-2 isoform is present in pancreatic beta cells.

Materials and Methods: A specific PFK-2 RACE PCR was performed with LD pancreatic islet cDNA. The sequences of the PCR fragments were obtained by the dideoxy-chain termination method. Homology plots were generated by FastA and NCBI-Blast2 searches (EBI) on the basis of GeneBank cDNA sequences of different PFK-2 isoforms from the rat. A full length rat islet PFK-2 cDNA was amplified from islet cDNA with specific primers coding for the brain isoform of rat PFK-2. Northern blot analyses were performed with total RNA from rat tissues.

Results: 5'-RACE and Northern blot analyses revealed that rat pancreatic islets express the brain isoform of PFK-2. A minor portion of the islet PFK-2 cDNA clones comprised a novel splice variant with five additional amino acids in exon 9 of the kinase domain. Northern blot analyses of various rat tissues with a PFK-2 0.9 kb cRNA probe cloned from rat pancreatic islets, which contained the additional intron sequence, revealed strong 4.2 kb and 2.1 kb hybridization signals in insulin-producing RINm5F cells, INS-1 cells and rat pancreatic islets. A distinct expression of the islet/brain PFK-2 mRNA could be also observed in brain, testis, heart and kidney, however, with a weaker intensity compared to insulin-producing cells. The binding of the islet/brain PFK-2 isoform to GK was comparable to that of the liver isoform. **Conclusion:** The interaction between GK and the bifunctional enzyme PFK-2 may provide the rationale for recent observations of a partial channeling of glycolytic intermediates between glucokinase and other glycolytic enzymes in dependence on the level of Fru(2,6)P₂. In pancreatic beta cells this interaction may have a regulatory function for the metabolic stimulus-secretion coupling for glucose-induced insulin secretion through changes of the Fru(2,6)P₂ level and for modulation of the brain type PFK-2 activity.

GAD65-mediated glutamate decarboxylation reduces glucose-stimulated insulin secretion in INS-1E beta cells and rat islets.

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Background: Mitochondrial metabolism plays a pivotal role in the pancreatic beta cell by generating signals coupling glucose sensing to insulin secretion. We have previously demonstrated that mitochondrially-derived glutamate directly participates in the stimulation of insulin exocytosis.

Methods: Our approach was to impose altered cellular glutamate levels by overexpression of glutamate decarboxylase (GAD) in order to repress elevation of cytosolic glutamate.

Results: INS-1E cells infected with a recombinant adenovirus vector encoding GAD65 showed efficient overexpression of the GAD protein with a parallel increase in enzyme activity. In control cells challenged with 15mM vs. 2.5mM glucose, there was a 2.3-fold increase in cellular glutamate levels. Upon GAD overexpression, glutamate concentrations were decreased both at 2.5mM (-36%, $p < 0.05$) and at 15mM glucose (-40%, $p < 0.02$). Insulin secretion was efficiently stimulated in control cells by 7.5 or 15mM glucose, and by 30mM KCl used to raise cytosolic Ca^{2+} levels. INS-1E cells overexpressing GAD exhibited impaired insulin secretion upon stimulation with 15mM glucose (-37%, $p < 0.05$), while the KCl response was preserved. In perfused rat islets, adenovirus-induced GAD65 overexpression reduced glucose-stimulated insulin release by 31% ($p < 0.05$).

Conclusions: GAD65-mediated glutamate decarboxylation activity in beta cells resulted in decreased glutamate levels and impaired glucose-evoked insulin secretion. These results are compatible with a role for glutamate as a glucose-derived factor participating in insulin exocytosis.

KATP channels at the nuclear membrane induce nuclear Ca^{2+} signals and gene expression.

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Background and Aims: Whilst the sequence of events linking glycemia with the activation of the exocytotic machinery is well described, the molecular mechanisms involved in glucose regulation of gene expression is still unknown. In spite of a substantial amount of information concerning localisation of Ca^{2+} related to the secretory process, little is known about Ca^{2+} signals located in other subcellular organelles. It is well established that the nucleoplasmic concentration of free Ca^{2+} regulates gene transcription, yet how the nucleoplasmic Ca^{2+} signals are generated, is still unclear. The aim of this study is to investigate the molecular pathway that links glucose metabolism with gene expression.

Materials and Methods: Mouse islet cells were cultured during 24 hours. Nuclei isolation was performed by brief cell sonication and centrifugation. Nucleoplasmic Ca^{2+} concentration was analysed with spot confocal and conventional confocal microscopy meanwhile nuclear KATP activity was measured with standard patch-clamp methods. Localization of sulphonylurea receptors was reported by glybenclamide-BODIPY labelling and cell identification by immunocytochemistry. C-Myc expression was evaluated using fluorescence in situ hybridization.

Results: We report here the existence of a glucose-regulated KATP channel at the nuclear envelope with similar properties to that on plasma membrane. The channel is sensitive to the specific blocker tolbutamide, which rises nuclear calcium in the nucleoplasm of isolated nuclei. We also demonstrate that these Ca^{2+} signals triggers C-myc expression in isolated cells.

Conclusions: This study provides the first demonstration of a functional KATP channel in nuclei linking glucose metabolism, nuclear Ca^{2+} rises and gene expression in pancreatic B-cells.

EXTRACTION OF FATTY ACIDS FROM RAT PANCREATIC ISLETS RESULTS IN A MASSIVE AUGMENTATION OF GLUCOSE-STIMULATED INSULIN SECRETION.

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Background and Aims: Fatty acids have complex effects in the control of insulin secretion. Acute exposure of islets to palmitate and other fatty acids increases insulin release. Chronic exposure to fatty acids inhibits secretion. To investigate the role of fatty acids further, we studied glucose-stimulated insulin release by rat pancreatic islets that had been depleted of fatty acids.

Materials and Methods: Rat pancreatic islets were isolated by collagenase digestion and incubated with 0.68% fatty acid-free BSA for up to four hours. Glucose-stimulated insulin release was measured under perfusion conditions and insulin measured by RIA. Palmitate and other fatty acids in the islets and in the extracting media were measured by HPLC and electrospray ionization mass spectrometry.

Results: 16.7 mM glucose-stimulated insulin release was enhanced up to ten-fold by four hours exposure to fatty acid-free BSA ($n=8$; $P < 0.01$). Both the first and second phases of release were enhanced as was the KATP channel-independent effect in the presence of KCl and diazoxide ($n=4$; $P < 0.01$). This massive responsiveness was associated with the removal of palmitate from the islets and its accumulation in the 0.68% BSA media. The enhanced response to glucose-stimulation was completely inhibited by 1 μ M norepinephrine. Exposure of islets during the incubation period with 0.68% BSA, to the general PKC inhibitor Ro 31-8220 abolished the response ($n=8$; $P < 0.01$) while Go 6976, which inhibits classical PKC isoforms, had no effect ($n=4$). Calphostin C, which inhibits the classical and novel isoforms, PKC μ and other DAG binding proteins, inhibited the response by 96% ($n=4$; $P < 0.01$). Inhibition of the response by cerulenin suggests that protein acylation is also involved.

Conclusions: These data suggest that the massive (ten-fold) enhancement of insulin secretion is dependent upon a novel isoform of PKC or one of the PKC μ /PKD type. The data demonstrate the importance of fatty acids, and palmitate in particular, to the control of insulin secretion. The massive enhancement of insulin secretion suggests the possibility of pharmacological or dietary intervention to markedly enhance glucose-stimulated insulin secretion.

T Two components of activity-dependent transient K^{+} -current (IKslow) in mouse pancreatic B-cells

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Background and Aims: The amount insulin secreted correlates with the time B-cells are electrically active. At intermediate glucose concentrations B-cells within intact islets generate bursts of action potentials which are rarely seen in dispersed B-cell, the standard preparation for patch-clamp experiments. We applied the patch-clamp technique to functionally identified B-cells within intact islets in order to identify the processes involved in the generation of the bursts of action potentials. **Material and Methods:** All experiments were performed in the perforated patch configuration on B-cells within intact pancreatic mouse islets. **Results:** Trains of simulated action potentials associated with the gradual development of an outward K^{+} -current (IKslow) with an average amplitude of 80 ± 28 pA ($n=10$). IKslow flowed through both sulphonylurea-sensitive TEA-resistant KATP-channels and sulphonylurea-resistant TEA-blockable K^{+} -channels. In the presence of tolbutamide (0.1 μ M), the peak current amplitude averaged 21 ± 3 pA ($P < 0.05$ vs. control). Upon cessation of electrical activity, this current deactivated with a biphasic time course. The kinetics of deactivation could be described as the sum of two exponentials with time constants (τ) of 0.9 s and 20 s. The rapid component was blocked by TEA (10 mM) whereas the slow component was abolished by tolbutamide. Increasing the glucose concentration from 10 to 20 mM reduced the slow (KATP-channel dependent) component by 50% whilst not affecting the peak amplitude. The Ca^{2+} -ATPase inhibitor thapsigargin increased electrical activity evoked by 10 mM glucose two-fold. This effect was associated with a 25% reduction of peak IKslow but there was no change in the holding current that could account for the stimulation of electrical activity. **Conclusions:** We propose that: 1) Ca^{2+} -entry during electrical activity leads to an increased K^{+} -conductance (IKslow) triggering membrane repolarization; 2) IKslow flows through two types of K^{+} -channel with distinct pharmacology, regulation and deactivation kinetics, one of which being identical to the KATP-channels; and 3) thapsigargin may stimulate electrical activity by exerting an ATP-sparing action by inhibition of the Ca^{2+} -ATPase thus preventing rapid reduction of the cytoplasmic ATP/ADP-level, opening of KATP-channels and membrane repolarization.

OP 13 Cell Biology of Smooth Muscle Cells

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GLUCOSE AND 12-LIPOXYGENASE REGULATE GLUT-1 mRNA STABILITY IN VASCULAR ENDOTHELIAL AND SMOOTH MUSCLE CELLS.

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Background and Aims: High glucose levels decrease the total cell content of GLUT-1 mRNA and protein in vascular endothelial and smooth muscle cells, thus, protect them against deleterious effects of increased intracellular glucose. We have previously shown that the 12-lipoxygenase (12-LO) product, 12-HETE, is involved in this mechanism. Indeed, 12-lipoxygenase expression and activity is increased in vascular cells exposed to hyperglycemic conditions. Inhibition of 12-LO results in increased levels of GLUT-1 mRNA and protein. The present study was aimed at identifying whether high glucose and 12-HETE affect the transcription and/or the stability of GLUT-1 mRNA. **Methods:** (1) Bovine aortic endothelial and smooth muscle cells were transfected with CAT expressing pGT1-1.3 plasmids under the control of enhancers 1 & 2 and the promoter of GLUT-1. The cells were exposed to various glucose concentrations in the absence or presence of LO inhibitors. (2) Various 3'UTR sequences of GLUT-1 mRNA were subcloned into the 3'UTR of the luciferase gene in PGL-2 promoter vector. Luciferase activity was measured following transfection of cells and incubation at various glucose concentrations without or with LO inhibition. (3) RNA Electro-Mobility Shift Assay (REMSA) and UV cross-linking with various 3'UTR sequences of GLUT-1 were used to study the interaction of proteins from vascular cells, exposed to various glucose levels and LO inhibitors. **Results:** (1) No evidence for glucose- or 12-LO-dependent transcriptional regulation of GLUT-1 in vascular cells was found. (2) However, at 25 mM glucose the nucleotide sequence 2098-2300 of GLUT-1 3'UTR reduced luciferase activity by ~30%. Treatment of these transfected cells with LO inhibitors reversed this effect and produced luciferase activity similar to that observed in vascular cell incubated at 5 mM glucose. (3) UV cross-linking and REMSA of this 3'UTR region showed interactions of specific cytoplasmic proteins, which are affected by pretreatment of cells with glucose and LO-inhibition. **Conclusions:** The regulation of glucose transport in vascular endothelial and smooth muscle cells by hyperglycemia and 12-HETE is directly related to corresponding changes of GLUT-1 mRNA levels in vascular cells. These changes do not result from transcriptional regulation of the GLUT-1 gene. However, this study shows a post-transcriptional regulation in GLUT-1 mRNA via specific interaction of cytosolic proteins, which interact with specific sequences of GLUT-1 mRNA 3'UTR. The interactions of these specific proteins seem to be regulated by glucose and HETEs.

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ROLE OF B2-PROTEIN KINASE C ON HYPERGLYCEMIA INDUCED INCREASE ON NITRIC OXIDE PRODUCTION

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Background and Aims: It is widely accepted that the development of vascular disease in diabetic patients is greatly accelerated by the coexistence of hypertension. Several clinical studies have consistently concluded that hyperglycemia is a primary cause of diabetic vascular complications. Moreover, changes on inducible nitric oxide synthase (iNOS) expression by interleukin-18 (IL-18) have been proposed to play an adaptive role in the vascular response to injury. Therefore, the goal of the present study was to determine whether hyperglycemia interferes on nitric oxide (NO) production and iNOS expression in IL-18-stimulated vascular smooth muscle cells (VSMC) from normotensive and hypertensive rats. **Materials and Methods:** Cultured VSMC from normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats were used. Cells were incubated with either 5 mM (control) or 22 mM (hyperglycemic conditions) glucose for 72 hours and stimulated with IL-18 (10 ng/ml) for the last 24 hours. Nitrite production by Griess reaction and iNOS expression by Western-blotting were determined. **Results:** Under normoglycemic conditions, IL-18 produced an increase on nitrite levels (WKY: from 11.8±1.6 to 64.4±8.9 nmol/mg protein, p<0.01; SHR: from 16.5±3.2 to 73.1±8.6 nmol/mg protein, p<0.01). Such effect was even higher when cells from WKY were submitted to hyperglycemic conditions (nitrite levels: from 64.4±8.9 to 95.2±14.5 nmol/mg protein, p<0.05). However, no effect by hyperglycemic conditions was observed in SHR VSMC. In accordance to this only VSMC from WKY stimulated with IL-18 showed an enhanced iNOS expression under hyperglycemic conditions. Hyperglycemia-induced effect on NO production and iNOS expression in WKY cells was abolished by the non-selective protein kinase (PKC) inhibitor calphostin C (1 µM) or the B2-selective PKC inhibitor LY379196 (30 nM). Nevertheless, both PKC inhibitors show no effect on cells from hypertensive rats. **Conclusions:** High glucose seems to increase induction of iNOS, and subsequently NO production, by activating the B2 PKC isoform in VSMC from normotensive animals. However, hyperglycemia does not affect iNOS expression levels in VSMC from hypertensive animals. This last result suggests that the induction of this enzyme by different stimuli may be altered in hypertension, which could reflect a loss of the adaptive role assigned to iNOS in the vascular response to injury in hypertension. (Supported by grants from DGICYT BXX2000-0153 and Lilly. LY379196 was a gift of Lilly Research Laboratories).

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EFFECTS OF GLUCOSE AND α-LIPOIC ACID ON GLUTATHIONE LEVELS, DNA DAMAGE AND APOPTOTIC PROTEINS IN SMOOTH MUSCLE CELLS.

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Introduction and aims: Oxidative stress plays a role in the pathogenesis of diabetic vascular complications; one element in this is intracellular glutathione (GSH) depletion. The aim of this study was to investigate the relationship between intracellular GSH, DNA damage and signs of apoptosis in vascular smooth muscle cells (VSMC) *in vitro*. **Methods:** VSMC were cultured in 5mM (NG), 25mM (HG) D-glucose or HG with the addition of α-lipoic acid (LA, 50µM) for 10 days. GSH was measured by a recycling assay. DNA damage was measured by the Comet assay. Nuclear morphology was assessed by Hoechst staining; expression of anti-apoptotic (Bcl-2, Bcl-xL) and pro-apoptotic (Bax, Bcl-xS) proteins was assessed by quantitative confocal microscopy. **Results:** GSH levels were decreased in HG and restored by the addition of LA (8.5±3.8, 3.8±1.6, 6.9±3.2 µmol/mg protein, NG, HG, HG+LA; NG vs HG, HG vs HG+LA, p<0.005, n=6). DNA damage was increased in HG (4.8±0.8% vs 17.0±1.8% tail DNA, NG vs HG, p<0.01, n=200) and reduced by the addition of LA (17.0±1.8%, 7.6 ± 1.0% tail DNA, HG vs HG+LA, p<0.001 n=200). VSMC cultured in HG did not show the nuclear characteristics of apoptosis. However, there was both an increase in Bcl-2 (NG vs HG, p<0.002, n=15) and Bcl-xL (NG vs HG, p<0.05, n=15) and a decrease in Bax (NG vs HG, p<0.005, n=24) and Bcl-xS (NG vs HG, p<0.001, n=16) in HG, ie increasing resistance to apoptosis. The addition of LA increased Bax (HG vs HG+LA, p<0.001, n=24) and Bcl-xS (HG vs HG+LA, p<0.001, n=16) as well as Bcl-2 (HG vs HG+LA, p<0.02, n=18) and Bcl-xL (HG vs HG+LA, p<0.002, n=14) in comparison to HG alone. **Conclusion:** Restoration of intracellular GSH by the addition of LA reduces glucose-induced DNA damage. VSMC survival is potentiated by increased expression of anti-apoptotic and decreased expression of pro-apoptotic proteins during glucose-induced oxidative stress.

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EFFECTS OF GLUCOSE AND FATTY ACIDS ON FREE RADICAL-INDUCED DAMAGE IN VASCULAR SMOOTH MUSCLE CELLS.

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Background and Aims: Diabetes represents a serious disturbance of homeostasis of glucose and fatty acid metabolism which may alter the cells' antioxidant defences. The aim of this study was to investigate the effects of high glucose and fatty acids on the antioxidant status and subsequent effects on free radical-mediated damage in porcine vascular smooth muscle cells (PVSMC) *in vitro*. **Methods:** PVSMC were grown in 5mM (NG) or 25mM (HG) D-glucose for 10 days. Physiological concentrations of linoleic acid (0.1mM) and α-linolenic acid (0.01mM) were added for the final 3 days of culture (BSA as carrier). **Results:** HG significantly decreased glutathione (GSH) levels (1.2±0.2 v 1.8±0.3 µM/mg protein, HG v NG) but had no effect on glutathione peroxidase (GPX), glutathione reductase (GR), catalase or total superoxide dismutase (SOD) activity. A significant increase in malondialdehyde (MDA, 561±53 v 354±20 pM/mg protein, HG v NG) and protein carbonyl formation (7.4±0.5 v 4.8±0.3 nM/mg protein, HG v NG) was observed in HG. Addition of α-linolenic acid significantly increased GSH levels (2.9±0.7 v 1.2±0.1 µM/mg protein, HG v BSA control) and catalase activity in HG (13.9±1.0 v 9.5±0.2 U/mg protein, HG v BSA control) but decreased GPX and GR activity. This increase in GSH was accompanied by a significant elevation in the mRNA expression of both subunits of γ-glutamylcysteine synthetase (rate-limiting enzyme in GSH synthesis). Linoleic acid had no effect on GSH levels, GPX, GR or SOD activity but significantly increased catalase activity (15.3±0.6 v 11.8±1.6 U/mg protein, HG v BSA control). The addition of linoleic acid also caused significant increases in both MDA (895±42 v 604±50 pM/mg protein, HG v BSA control) and protein carbonyl formation (9.6±0.2 v 7.3±0.3 nM/mg protein, HG v BSA control) in HG. By contrast, α-linolenic acid significantly reduced MDA (472±23 v 582±38 pM/mg protein, HG v BSA control) and protein carbonyl formation (5.0±0.2 v 6.5±0.04 nM/mg protein, HG v BSA control) in PVSMC. **Conclusions:** Linoleic acid increases free radical-mediated damage in PVSMC whereas α-linolenic acid decreases the oxidative damage in both glucose concentrations. These results have considerable implications in the treatment of diabetes whereby supplementation with α-linolenic acid may increase antioxidant defences and reduce free radical damage in the vasculature.

GLUCOSE-POTENTIATED VASCULAR SMOOTH MUSCLE CELL CHEMOTAXIS IS MEDIATED BY PHOSPHOINOSITIDE-3-KINASE-P110 β
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Background: In atherosclerosis, migration and chemotaxis of vascular smooth muscle cells (VSMCs) play a major role in lesion formation. VSMCs exposed to 5mM glucose do not show chemotaxis in a gradient of foetal calf serum (FCS) without prior serum starvation, while those exposed to 25mM glucose (HG) show chemotaxis. Phosphoinositide-3-kinase (PI3K) has been shown to mediate migration in a number of cell types, and could play an important role in regulating the glucose-induced changes in VSMC migration. The aim of this study was to determine the role of PI3K in mediating altered VSMC chemotaxis in HG. **Methods:** Neutralising antibodies specific to the α , β and δ isoforms of the p110 catalytic subunit of PI3K were microinjected into human VSMCs, which were then placed in a gradient of FCS within the Dunn chemotaxis chamber in HG. As controls, VSMCs were injected with a non-specific rabbit IgG; and additionally each neutralising antibody was microinjected following incubation with a high molar excess of a cognate competitive binding protein. The effects of inhibiting each of the p110 isoforms on the actin cytoskeleton were investigated by TRITC-phalloidin staining. **Results:** The PI3K inhibitor LY294002 blocked VSMC chemotaxis in HG. Microinjection with non-specific rabbit IgG or with antibodies against p110 α and p110 δ had no effect on chemotaxis. In contrast, microinjection of the anti-p110 β antibody inhibited chemotaxis. No inhibition was observed when the anti-p110 β antibody was incubated with its cognate peptide prior to microinjection. Actin staining revealed no changes in the VSMC cytoskeleton following microinjection of any of the antibodies. **Conclusions:** Elevated glucose sensitises VSMCs to serum factors, inducing chemotaxis via a PI3K-p110 β mediated signal pathway. Therefore p110 β could be a potential target for new therapeutic approaches to reduce the risk of atheroma in diabetes.

PLATELET DERIVED GROWTH FACTOR-B INDUCED CHEMOTAXIS IN VASCULAR SMOOTH MUSCLE CELLS: GLUCOSE POTENTIATION

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Background: Diabetic subjects are noted to have premature onset and increased severity of atherosclerosis. In atheroma formation a subpopulation of vascular smooth muscle cells (VSMCs) migrate from the tunica media into the sub-endothelial area. This study investigated the effects of elevated concentrations of D-glucose on the migratory behaviour of VSMCs and its regulation, using the Dunn chemotaxis chamber assay. **Methods:** Foetal calf serum (FCS) or Platelet derived Growth Factor-B (PDGF-B) was used as the chemoattractant. VSMCs generated from primary explants were cultured in 5 mM D-glucose and exposed to higher glucose only when placed within the chemotaxis chamber. **Results:** No chemotaxis occurred at 5 mM D-glucose in a FCS or PDGF-B gradient, unless preceded by 24 hours serum starvation. By contrast, positive chemotaxis occurred in both a FCS and a PDGF-B gradient at glucose concentrations ≥ 9 mM ($p < 0.01$, Rayleigh test, for both). High glucose (25mM) induced a 2 fold increase in PDGF-B receptors (quantitative confocal microscopy, $n=10-20$; and immunoprecipitation). Pre-incubation with an anti-PDGF- β receptor neutralising antibody inhibited chemotaxis. Both the PI(3)kinase inhibitor wortmannin and the MAPKinase inhibitor PD 98059 blocked chemotaxis in human and porcine cells. The PKC inhibitor H7 blocked chemotaxis in human VSMCs, and reduced speed of porcine cells without affecting directionality of movement. **Conclusions:** This study demonstrated that mild, short term rises in D-glucose concentration can increase VSMC sensitivity to chemotactic factors in serum, especially PDGF, leading to altered migratory behaviour *in vitro*. It is probable that similar processes occur *in vivo* and that glucose-enhanced chemotaxis of VSMCs mediated through the PDGF- β receptor-operated pathways involving PI(3)K, MAPK and PKC contribute to the accelerated formation of atheroma in diabetes in man. Therefore PI(3)K, MAPK and PKC could be potential targets for new therapeutic approaches to reduce the risk of atheroma in diabetes.

OP 14

Type 2 Diabetes – Genetics: Candidate Gene Studies

The function and survival of isolated human islets carrying the Arg972 IRS-1 polymorphism.

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Background and Aims: The common Gly to Arg972 polymorphism of IRS-1 (Arg972 IRS-1) has been associated with human Type 2 diabetes and low insulin and C-peptide plasma levels. In transfected beta-cells Arg972 IRS-1 impairs cell function and survival. In the present study we prepared isolated human islets (ISLs) from human donors carrying the Arg972 IRS-1 polymorphism and evaluated several of their properties. **Materials and Methods:** ISLs were prepared by collagenase digestion and density gradient purification and challenged them with varying glucose concentrations (1.7 to 16.7 mM) and other secretagogues; glucose oxidation and utilization were assessed by labeled glucose techniques; electron microscopy was used to assess beta-cell morphology; cell death was measured by flow cytometry and an ELISA method. **Results:** Insulin content (IC) was lower in variant than in control ISLs (94 \pm 47 vs 133 \pm 56 μ U/islet, mean \pm SD, $p < 0.05$). Stepwise glucose increase significantly potentiated insulin secretion (IS, % of IC) from control but not Arg972 IRS-1 ISLs, which had a 33% lower release at 16.7 mM G (2.9 \pm 1.1 vs 4.3 \pm 1.4%, $p = 0.05$). In addition, the variant ISLs showed a slightly lower response to glibenclamide (3.5 \pm 2.4 vs 4.5 \pm 1.6%), and a significantly higher response to arginine (4.7 \pm 1.3 vs 2.6 \pm 0.6%, $p < 0.05$). Proinsulin secretion mirrored that of insulin and glucagon release was similar in Arg972 IRS-1 and control ISLs. Glucose oxidation and utilization did not differ in variant and wild-type ISLs at any tested glucose level. Electron microscopy showed that Arg972 IRS-1 beta-cells had a significantly higher amount of immature secretory granules (3.3 \pm 0.7 vs 0.5 \pm 0.2 ml%, $p < 0.05$), and a lower amount of mature granules (0.9 \pm 0.2 vs 3.6 \pm 0.7 ml%, $p < 0.05$) than control beta-cells. Flow cytometry and ELISA techniques showed a two-fold higher amount of apoptotic beta-cells in Arg972 IRS-1 than control ISLs. **Conclusions:** Arg972 IRS-1 human ISLs have lower insulin content, impaired glucose-stimulated insulin secretion, more immature secretory granules, and enhanced beta-cell apoptosis. These alterations can account for increased predisposition to Type 2 diabetes in individuals with the Arg972 IRS-1 polymorphism.

Association of T786C mutation of the Endothelial Nitric Oxide Synthase Gene with Insulin Resistance and Visceral Fat Obesity

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Background and Aims: Expression of endothelial NO synthase (eNOS) and NO production in subcutaneous tissue are known to decrease in obese human subjects. Knockout of eNOS gene results in insulin resistance. Whether impairment of endothelial NO production leads to insulin resistance / obesity remains to be clarified in human subjects. **Materials and Methods:** To establish the association between the NO production and obesity / insulin resistance, we evaluated the relationship between the two polymorphisms (T786C mutation in the 5'-flanking region and G894T mutation in exon7) of eNOS gene and clinical characteristics of 144 healthy non-diabetic Japanese men and 42 early type2 DM patients. Glucose infusion rate (GIR) was evaluated by hyperinsulinemic euglycemic clamp in type 2 diabetic patients. Abdominal fat distribution was assessed by abdominal fat index (AFI) using ultra sonography. Genoms are obtained from leukocytes and genomic analysis was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). **Results:** Allele frequencies are similar between non-diabetic subjects and type2 diabetic patients (786TT(n=120) / 786TC+CC (n=21+3) in healthy men vs 786TT(n=34) / 786TC+CC(n=7+1) and 894GG(n=124) / 894GT+TT(19+1) in healthy young men vs 894GG(n=34) / 894GT+TT(n=8+0)) In non-diabetic men, fasting IRI, Body mass index (BMI) (\pm SD) and AFI is significantly higher in 786TC+CC groups (F-IRI; 9.2 \pm 2.5 vs 7.8 \pm 2.5 uU/ml, BMI; 24.2 \pm 3.2 vs 22.9 \pm 2.3 kg/m², AFI; 1.40 \pm 0.57 vs 0.89 \pm 0.36, $p < 0.05$). HOMA-R tends to be higher in 786TC+CC groups (2.11 \pm 0.67 vs 1.81 \pm 0.61, $p = 0.063$). In type2 diabetic patients GIR is significantly lower in 786TC+CC groups (4.37 \pm 1.05 vs 5.59 \pm 1.83 mg/kg/min). On the contrary, G895T mutation of eNOS gene shows no significant difference in the clinical characteristics of non-diabetic men and type2 DM patients. **Conclusions:** These results indicate that T786C mutation in the 5'-flanking region of eNOS gene is weakly but significantly associated with obesity, especially with visceral fat obesity and also with insulin resistance at least in non-diabetic and diabetic Japanese subjects.

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A polymorphism of the CD14 Monocyte Receptor Gene, involved in the inflammatory cascade, is associated with insulin sensitivity

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Background and Aims: Lipopolysaccharide (LPS) is the one of the most potent biologic response modifiers currently recognized. Soluble CD14 (sCD14), detectable at high concentrations constitutively present in the circulation, plays a key role as intermediate in the neutralization of LPS under physiological conditions. A polymorphism of the CD14 gene, a C-to-T transition at nucleotide -159 from the major transcription start site, seems to play a significant role in regulating serum sCD14 levels.

Patients and Methods: We evaluated insulin resistance, inflammatory and endothelial markers in apparently healthy individuals and in patients with type 2 diabetes mellitus in relation to CD14 gene polymorphism.

Results: Healthy subjects (n=60) homozygotes for the C allele, associated to lower circulating levels of sCD14 in a recent study, were similar in age, sex, body mass index, fat mass, waist/hip ratio, blood pressure, and fasting glucose and insulin levels in comparison with carriers of the T allele. The integrated area under the curve of serum glucose concentrations after OGTT (AUC glucose) was significantly higher (p=0.02) in C/C healthy subjects in the presence of nonsignificantly different integrated insulin levels, and also showed a significantly lower insulin sensitivity index than carriers of the T allele, as measured during an oral glucose tolerance test (p=0.033) or using the frequently sampled intravenous glucose tolerance test with minimal model analysis (p=0.036). Type 2 diabetic subjects (n=33) who were C/C homozygotes were also similar in age, sex, years of evolution of diabetes, body mass index, waist-to hip ratio, usual metabolic control, type of antidiabetic treatment and chronic diabetic complication in comparison with carriers of the T allele. C/C type 2 homozygote patients also showed a significantly lower insulin sensitivity index than carriers of the T allele (p=0.03), and had significantly increased circulating sICAM-1 levels (p=0.01).

Conclusions: To our knowledge, this is the first study to demonstrate an effect of a genetic polymorphism on both insulin sensitivity and endothelial dysfunction in type 2 diabetes mellitus.

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THE 3'UTR POLYMORPHISM OF THE LEPTIN RECEPTOR GENE IS ASSOCIATED WITH ELEVATED INSULIN LEVELS AND TYPE 2 DIABETES

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Background and Aims: We have previously identified Lys109Arg and Gln223Arg polymorphisms in the leptin receptor gene (LEPR) and studied their role in the pathogenesis of type 2 diabetes and leptin resistance within the Botnia study. The aim of the present study was to investigate whether the LEPR 3'UTR insertion (I)/deletion (D) polymorphism increases susceptibility to type 2 diabetes or affects insulin and leptin levels in patients with and without type 2 diabetes. **Materials and Methods:** The 3'UTR polymorphism was genotyped by PCR and gel electrophoresis in 300 (128 males/172 females, age 53±15 years, BMI 27±5 kg/m², FBG 4.9±0.5 mmol/l, mean±SD) non-diabetic subjects and in 390 (177 males/213 females, age 65±10 years, BMI 29±4.7 kg/m², FBG 8.6±2.6 mmol/l) patients with type 2 diabetes from the Botnia region in Finland. **Results:** The I-allele was in linkage disequilibrium with allele Gln223 and allele Lys109. The allele frequency of the I-allele did not differ between type 2 diabetic and non-diabetic subjects (13.5% vs. 12.5%, p=0.59). However, the II-genotype was significantly more common among the type 2 diabetic patients (0.56% vs. 3.58%, p=0.038). Age, gender, BMI and FBG did not differ significantly between the different genotype carriers. However, the non-diabetic carriers of genotypes I/D and I/I had higher insulin levels at 120 minutes (OGTT) (63.8±55.1 vs. 52.05±53.1 mU/l, p=0.009) and higher S-leptin concentrations (17.5±15.6 vs. 13.9±13.7 ng/ml, p=0.023) compared to genotype D/D carriers. The differences in insulin and leptin levels were not statistically significant between diabetic carriers of I/D and D/D genotypes (80.9±67.9 vs. 64.6±52.1 mU/l, p=0.22 and 18.0±14.7 vs. 14.7±12.4 ng/ml, p=0.08). Multiple forward stepwise regression analysis suggested S-leptin (p<1x10⁻⁶), BMI (p<1x10⁻⁶), age (p<1x10⁻⁶), glucose 120 (p<1x10⁻⁵), gender (p<1x10⁻⁴) and the LEPR genotype (p=0.006) as independent predictors for increased insulin concentrations at 120 minutes. The LEPR genotype did not significantly affect S-leptin when gender (p<1x10⁻⁶), BMI (p<1x10⁻⁶) and fasting S-insulin (p<1x10⁻⁶) were included in the model (p=0.40). **Conclusions:** Our results indicate that the LEPR 3'UTR I-allele is associated with elevated insulin values and the I/I genotype with increased risk for type 2 diabetes.

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POSITIONAL CANDIDATE GENE SCREENING OF A LOCUS ON CHROMOSOME 18p11 LINKED TO TYPE 2 DIABETES WITH OBESITY

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Background and Aims: Our recent genome-wide linkage analysis indicated excess allele sharing on chromosome 18p11 among a subgroup of obese type 2 diabetes sib pairs (LOD 4.22). In the present study, seven candidate genes on 18p11 were screened for mutations and single nucleotide polymorphisms (SNPs) in 64 families contributing to the original linkage result. **Materials and Methods:** One obese patient with type 2 diabetes from each of the 64 families (m/f 26/38, Age 63±9 y, BMI 35.6±4.2 kg/m²), of which 38 originated from families supporting linkage with a family NPL-score >1 (mean 1.5; set 1) and 26 from families with a NPL-score <1 (mean 0.3; set 2) in or near the linked region, was mutation screened by SSCP and sequenced. Allele frequencies of the identified SNPs were compared between set 1 and 64 nondiabetic lean controls (m/f 31/33, Age 61±9 y, BMI 23.7±2.1 kg/m²) for association to diabetes with obesity, and between set 1 and set 2 for evidence of association to the linkage result. **Results:** Altogether 21 SNPs but no obvious functional mutations were identified in the genes coding for the NADH ubiquinone dehydrogenase (NDUFV2), the olfactory type guanine nucleotide binding protein (GNAL), the translation initiation factor EIF4A2, the pituitary adenylate cyclase activating polypeptide (PACAP), the melanocortin receptors 2 and 5 (MC2R, MC5R) and the transcription factor GATA6. A common C-allele of one SNP located next to the transcription initiation site of the MC2R gene was significantly more common among obese type 2 diabetes patients (set 1) compared to controls (98.6% vs 89.1%, p=0.014) and to set 2 (80.8%, p=0.0006). Allele frequencies of the C-allele were similar between Finnish and Swedish subgroups (set 1; 97.6% vs 100.0%, controls; 87.5% vs 91.7%). **Conclusions:** We provide evidence that a variant in the MC2R promoter is associated with obese type 2 diabetes and propose the MC2R/MC5R gene cluster as a prime target for further linkage disequilibrium mapping of this putative diabetes locus.

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Interaction effect between common polymorphisms in PPARγ (Pro12Ala) and IRS-1 (Gly972Arg) on insulin sensitivity

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Background and Aims: The Pro12Ala polymorphism in the peroxisome proliferator-activated receptor (PPAR) γ2 gene is associated with a reduced risk for type 2 diabetes. A beneficial effect on insulin sensitivity was reported in some but not all populations. It is possible that this genetic variant produces a characteristic phenotype only before a certain genetic background. The aim was to test the hypothesis that carriers of the Ala allele of PPARγ exhibit a different phenotype before the background of the Gly972Arg polymorphism in the insulin receptor substrate (IRS)-1. **Materials and Methods:** We determined insulin sensitivity in 4 haplotypes defined by absence or presence of polymorphic allele (healthy, glucose tolerant subjects) by oral glucose tolerance test (OGTT) (using a validated index, N = 318) and hyperinsulinemic clamp (N = 201).

Results: Insulin sensitivity was not (215 ± 6 vs 221 ± 11, p = 0.67; OGTT) or only marginally (12.1 ± 0.4 vs 13.6 ± 0.08, p = 0.8; clamp) different between Pro/Pro and X/Ala in the whole population. Interestingly, insulin sensitivity was significantly greater in X/Ala (PPARγ) + X/Arg (IRS-1) (299 ± 30 units) compared to Pro/Pro (PPARγ) + X/Arg (IRS-1) (202 ± 16 units, p = 0.01) using the OGTT index. On the other hand, insulin sensitivity was similar in the X/Ala (PPARγ) + Gly/Gly (IRS-1) (206 ± 11 units) and the Pro/Pro (PPARγ) + Gly/Gly (IRS-1) (218 ± 6, p > 0.10). The results were practically identical using insulin sensitivity from the clamp.

Conclusions: The Arg972 (IRS-1) background produced a marked difference in insulin sensitivity between X/Ala and Pro/Pro (PPARγ) which was not present in the whole population or before the Gly972 (IRS-1) background. This suggests that the Ala allele of PPARγ becomes particularly advantageous before the background of an additional, possibly disadvantageous genetic polymorphism. Allowing for gene-gene interaction effects may reveal novel information regarding metabolic effects of genetic variants.

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Diabetic Foot: Clinical/Epidemiology

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REACHING THE SAINT VINCENT TARGETS STEPWISE: DIABETIC AMPUTATION AND ULCER RECURRENCE FROM A GERMAN SETTING
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Background and Aims: In 1989, the Saint Vincent Declaration targeted a 50% reduction of lower extremity amputations among diabetics within a five year period to improve patient care across Europe. The most effective way to avoid amputation is to reduce the incidence of initial ulceration and reulceration. We therefore investigated the changes of outcome and recurrence of diabetic foot lesions over time at a specialized setting. **Patients and Methods:** Between 1994 and 2000, 1522 patients with diabetic-foot-syndrome were treated at our diabetic foot clinic. 1.) To evaluate the changes of outcome over time we compared treatment results of patients with a history of ulceration prior to initial presentation to our foot clinic (Group A, n=312) with the outcome of patients receiving structured treatment at our setting between 1994 and 1996 (B, n=386), and between 1999 and 2000 (C, n=442). 2.) To evaluate the efficacy of tertiary prevention, we furthermore compared the recurrence rates during the first year after healing in all patients who initially contacted our service with an ulceration in 1994/95 (B2, n=107) and in 1999 (C2; n=137). **Results:** Without structured therapy, 53.8% of the cases were treated with amputation (Group A, Minor: 33.3%; Major: 20.5%). During the initial years of our foot service, the total amputation rate decreased to 30.6 % (B, Minor: 17.9%; Major: 12.7% - $p<0.001$) with a further reduction to 17.4% during the time period 1999/2000 (C, Minor: 11.5%; Major: 5.9% - $p<0.001$ vs B). The corresponding reulceration rates after one year were 33.6% (B2) and 14.6% (C2 - $p<0.001$). **Conclusions:** By implementing outpatient structures of care and inpatient facilities for the diabetic foot patient using a multidisciplinary approach, an amputation reduction of approximately 70% is attainable. Through continuous education of all team members including the patient at risk and his relatives, the rate of reulceration can also be lowered significantly.

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Foot health education and its impact on the occurrence of foot ulcers in diabetes.
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Background and Aims: Patient education is considered to be an important feature in the prevention of diabetic foot ulcers, but the impact that an education program has on the prevention of foot complications in diabetes patients is yet to be fully explored. This study examined data from two large groups of diabetic patients drawn from the North West of England.

Materials and Methods: The first group (Group 1, n=9710) underwent a baseline foot examination and also completed a knowledge questionnaire covering aspects of foot inspection, footwear, prevention and treatment of foot problems. Those showing deficiencies in knowledge or understanding in preventive foot care were given structured advice provided by a podiatrist or diabetes specialist nurse. Follow up questionnaires were sent to all Group 1 subjects after 2 years with 6613/9710 (68%) responding. After rigorous crosschecking, the occurrence of self-reported diabetic foot ulcers was compared with a second group (Group 2, n= 5982) who were recruited and examined at the same time as the Group 1 follow up, but did not have the structured advice.

Results: Both groups were similar in age (ttest $p>0.79$) and gender (Chi-squared $p=0.93$). The occurrence of past (over the preceding two years) or current foot ulcers was 4.3% in Group 1 subjects and 5.3% in Group 2 subjects representing a small but significant ($p=0.01$) decrease in occurrence of foot ulcers amongst those receiving the structured advice (Odds Ratio 0.81 95% CI 0.68,0.95).

Conclusions: These findings provide some evidence that a large-scale structured education and examination program, over and above that received in a usual clinical setting, may prove beneficial in reducing the occurrence of foot ulcers in diabetic patients over a two-year outcome period. Further research on the benefits of more frequent education programmes (including foot health and glycaemic control) in the general diabetes population is required to determine whether this may improve the outcomes.

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DO DIABETOLOGISTS ADEQUATELY DETECT PATIENTS AT RISK FOR AMPUTATION?

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Background and Aims: Earlier studies have reported a relative large patient delay before medical care for diabetic foot ulceration is sought. Little is known about the doctor's delay. Foot ulceration and peripheral ischemia are major risk factors for amputation. "Podoproof" is a three year randomised multicenter trial with the aim to determine if podiatric care prevents foot ulceration in diabetic patients with polyneuropathy (PNP). The inclusion phase of Podoproof enabled us to gain insight to which extent diabetologists correctly categorise patients as being at risk for future ulceration or amputation. **Materials and Methods:** Diabetologists in 12 Dutch hospitals were asked to include diabetic patients with clinical polyneuropathy in the study and to fill in a simple questionnaire with 9 items when a patient visited the clinic. These questions included amongst others items on: the presence of an active foot ulcer; a healed ulcer during the past year; previous vascular surgery in the lower legs; presence of severe ischemia; podiatric care in the past year. Any "Yes" meant exclusion from the project. Within 2 weeks patients were screened by a podiatrist: foot inspection, Semmes Weinstein monofilament (10 gram), vibration sensation with a tuning fork (128 Hz) and ankle blood pressure (Doppler). **Results:** 2420 patients were referred by the diabetologists and were screened. An active foot ulcer, despite the belief of the diabetologist that such an ulcer was not present, was found in 63 (3.6 %) patients. Severe ischemia (Ankle pressure <50 mmHg) was present in 42 (2.4%) patients. In total 105 patients (5%) had very high risk for amputation, undetected by the diabetologist. In 222 (12.6 %) patients no signs of neuropathy (with the 2 tests used) were observed, despite the clinical diagnosis of polyneuropathy. In 225 (12.7%) cases the diabetologist was not aware that the patient had regular podiatric care. **Conclusions:** Our data suggest that diabetologists tend to underestimate important risk factors for future amputation. Although the number of patients with undetected foot ulceration or severe ischemia was not large (5%), on a population basis this unawareness could result in relative large numbers of preventable amputations.

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Ethnic Variations in Diabetic Foot Ulcer Risk, Neuropathy and Peripheral Vascular Disease.

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Background and Aims: There are very few data regarding prevalence of diabetic foot ulcers, peripheral neuropathy or peripheral vascular disease in different ethnic groups. We therefore performed a large, population-based study of diabetic patients in six districts of North West England, an area of high ethnic diversity, to examine potential ethnic differences in risk factors for foot ulcers.

Materials and Methods: All adult diabetic patients were targeted for screening in a diabetic footcare program at various healthcare settings in the community. After two years 9,710 diabetic patients had been screened for foot ulceration, foot deformities, measures of neuropathy and peripheral vascular disease (PVD), plus other medical and social characteristics. Patients' ethnicity was defined as White Caucasian (WC) (n=8,508; 87.6%), South Asian (SA) (n=920; 9.5%), African Caribbean (AC) (n=260; 2.7%) and Other (n=22; 0.2%).

Results: SA patients were younger than WC and AC (55.4 vs. 61.9 and 61.9 years, respectively, $p<0.0001$) and had a shorter diabetes duration (7.1 vs. 8.4 and 8.7 years, respectively, $p<0.0001$). The prevalence of diabetic foot ulcers (past or present) for WC, AC and SA was 5.2%, 3.1% and 1.5% ($p<0.0001$, AC vs WC). Furthermore, SA had lower prevalences of neuropathy, PVD, foot deformities, smoking, and alcohol consumption than WC ($p<0.0001$), whereas for AC, only smoking rate and vibration sensation was reduced compared to WC ($p<0.0001$). The unadjusted risk of ulcer between the ethnic groups was significant for SA vs WC only [odds ratio (OR) = 0.29 (0.17-0.49, 95% CI), $p<0.0001$]. Adjustment for age and diabetes duration in the logistic regression model did not affect the SA ulcer risk reduction appreciably [OR = 0.33 (0.22-0.65, 95% CI), $p<0.0001$]. Adding PVD, neuropathy, smoking and foot deformities into the model attenuated the age-adjusted OR from 0.33 to 0.54 (95% CI 0.31, 0.94, $p=0.029$), however the reduced ethnic ulcer risk in SA was still significant. **Conclusions:** Although the reduced prevalence of neuropathy, peripheral vascular disease and smoking in diabetic South Asians accounts partly for their reduced ulcer rate, there are other, as yet undetermined, reasons for fewer ulcers. These may be linked to dietary or genetic variations in ethnicity.

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TREATMENT STRATEGIES AND AMPUTATION RATES IN ADVANCED DIABETIC FOOT DISEASE IN A TERTIARY FOOT CARE CENTRE

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Background and aims: Patients with diabetic foot lesions complicated by bone infection and/or ischemia are at high risk for major amputation. We evaluated a multidisciplinary, strictly sequential therapeutical approach with infection control, revascularisation and selective osteotomy. **Materials and methods:** 102 consecutive patients with diabetic foot wounds admitted for inpatient treatment between October 1999 and September 2000 were studied. MR-imaging was used to confirm osteomyelitis. We applied the University of Texas (UT) Wound Classification (Armstrong, 1998) to characterize foot lesions according to depth and affected tissues (Grade 0-III) and the prognostic factors infection and peripheral arterial occlusive disease (Stage A-D). **Results:** 21,6% were classified UT IIIB (Bone involvement and infection), 7,8% IIIC (bone involvement and ischemia) and 56,9% IIID (bone involvement, ischemia and infection). Bypass grafting was done in 50,0% of all patients, with a pedal vessel as distal origin in 45,1% of all bypasses. Minor surgery on the level of the forefoot was carried out in 53,9% of patients, forefoot-amputations in 7,8%, Syme-amputations in 2,9% and higher level amputations in 5,9% of cases. The rate of minor amputation did not increase within UT grade III (IIIB 68,2%, IIIC 75,0%, IIID 57,6%). The frequency of major amputation was low (6,9%) even in the most serious category IIID (IIIC 12,5%, IIIB 0%). Severe concomitant disease (chronic heart failure, Parkinson's disease) and poor general condition were the main factors in the decision against limb salvage. **Conclusions:** Even in advanced stages of diabetic foot disease high limb salvage rates can be accomplished by a multidisciplinary approach. The low rate of higher level amputations despite a high incidence of UT IIID-lesions reflects the important role of surgical revascularisation. In this context comorbidity rather than osteomyelitis or ischemia remains the limiting factor in the strive against amputation.

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A multicenter, randomized controlled clinical trial to evaluate the efficacy of hyaluronan based dermal and epidermal autologous grafts in the treatment of diabetic foot

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Background and Aims: Foot ulcers are a major worldwide health problem and the major cause of amputation in patients with diabetes.

Materials and Methods: A randomized controlled study was undertaken to determine if a two stage hyaluronan based autologous graft procedure (Hyalograft 3D, Laserskin -Fidia Advanced Biopolymers, srl, Italy) could promote healing of these ulcers. Ethical committee approval was obtained from all centers. Patients with type 1 and type 2 diabetes with an ulcer > 2 cm², a transcutaneous oxygen pressure > 30 mmHg and Ankle arm index > 0.5 were enrolled. Standard care including debridement, infection control and off-weight bearing methods (fiberglass off-loading cast for plantar ulcers) was given to all ulcers regardless of treatment group. Patients were randomized to either hyaluronan based autologous graft group or control group (conventional therapy). Patients attended weekly control visits for a total of 11 weeks. A total of 82 patients were enrolled. Thirty-seven patients had dorsal ulcers and 45 plantar. At baseline the two groups were comparable. The average ulcer size was 6.2 cm² for graft group and 5.3 for control group.

Results: The percent of ulcers healed was steadily higher in the hyaluronan based autologous graft group than in the control group (65% Vs 41.7% at week 11). The estimated time to complete healing was 55 Vs. 77 days for the hyaluronanbased autologous group and the control group, respectively. The difference between the percent of dorsal ulcer healed was statistically significant (65% Vs 31.3% p=0.044). Furthermore 20% of ulcers healed by week 4 in the graft group and 0% in the control group. Considering plantar ulcers no difference in percent of ulcers healed and in time to healif healing was observed. No adverse event related to the study treatments was recorded.

Conclusion: These successful results demonstrate that this novel technique using hayluronan based dermal and epidermal autologous graft is a valid alternative for the treatment of diabetic foot ulcers. Regarding plantar ulcers data from the study show that the use of off-loading cast, wich has to be considered the standard care for the treatment of these ulcers, makes not useful the application of dermal substitutes to obtain an increase in percent of ulcers healed and reduction of time to healing.

OP 16

Issues in Insulin Treatment

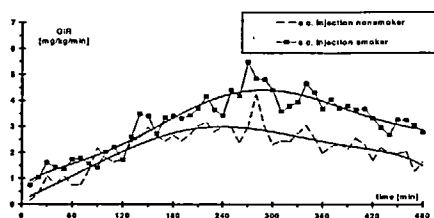
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IMPACT OF SMOKING ON THE METABOLIC ACTION OF SUBCUTANEOUS REGULAR INSULIN IN TYPE 2 DIABETIC PATIENTS

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Background and Aims: In Type 2 diabetes, smoking has been repeatedly associated with hyperinsulinaemia, which is supposed to be the consequence of smoking-induced insulin resistance. However, as the pharmacokinetic (PK) and pharmacodynamic (PD) properties of regular insulin (RI) has never been compared between smoking (SM) and non-smoking (NSM) Type 2 diabetic patients, it is still unclear whether smoking deteriorates insulin clearance rather than insulin sensitivity. **Material and Methods:** 9 SM (≥ 10 cigarettes per day) and 9 NSM matched for gender, age, and BMI (without significant differences in HbA_{1c}, diabetes duration and blood pressure) were enrolled in the study. Patients' blood glucose was stabilized overnight at 7.2 mmol/l by means of the euglycemic glucose clamp technique. SM were required to smoke one cigarette within 1.5 hours prior to injection of 18 U RI s.c. in the morning. Glucose infusion rates (GIR) were registered for the subsequent 480 min. **Results:** Injection of 18 U of RI resulted in significantly higher insulin concentration in SM compared to NSM: Insulin-AUC₂₄₀₋₄₈₀ (32±9 vs. 24±6 µU/ml, p=0.036). Consequently, SM showed a higher metabolic effect compared to NSM with significant differences in the last four hours of the experiment (AUC₂₄₀₋₄₈₀ 925±403 vs. 405±154 mg/kg, p=0.047). **Conclusion:** Our results suggest that hyperinsulinaemia in smoking Type 2 diabetic patients might be caused by a decrease in insulin clearance rather than in insulin sensitivity.



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EFFECT OF ADEQUATE VS INADEQUATE RESUSPENSION OF NPH INSULIN PRIOR TO S.C. INJECTION ON PHARMACOKINETICS AND -DYNAMICS IN T1DM

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Although it is well known that NPH insulin should be adequately resuspended prior to s.c. injection, patients not always do so especially when using pens. Aim of the present study was to assess the pharmacokinetics (PK) and -dynamics (PD) of s.c. pen injection of NPH after adequate resuspension (tipping the pen up and down for 15 times) (Study I, SI), or inadequate resuspension [no tipping, pen immobilized in vertical position for 12 hours with the needle up (SII), or down (SIII)]. Eight T1DM patients were studied at 2-3 week intervals in random order after s.c. injection of 0.3 U/kg in the internal part of one thigh with the previously described isoglycemic glucose clamp technique (Diabetes 49:2142-8, 2000). Onset of action was assumed as time required to decrease basal i.v. insulin needs to maintain PG at 130 mg/dl after s.c. NPH injection; end of action was time of increase of BG>150 mg/dl in the absence of glucose infusion; duration of action was end minus onset of action. AUC is area under glucose infusion curve.

	Study I	Study II	Study III
Onset of action (h)	0.75±0.18	1.01±0.22	0.51±0.13
End of action (h)	15.5±2.1	11.5±1.9	19.5±2.3
Duration of action (h)	14.7±2	10.5±1.8	19±2.2
Gmax (mg/kg/min)	2.23±0.4	1.47±0.3	2.8±0.3
Tmax (h)	4.7±0.39	3.6±0.4	6.2±0.7
AUC (mg/kg/G infusion time)	15.5±1.9	9.7±1.2	24.7±2.9

(SI vs SII vs SIII, all p<0.05). Plasma insulin dynamics paralleled the GIR. As compared to adequate resuspension (SI), absent resuspension results in major differences in PK and PD. The effect of NPH is greater after injection of the cloudy, insulin crystal reach part and may differ up to 2.5 times from injection of the less cloudy, insulin crystal poor, part. These differences may play a role in the day-to-day variability of BG control in T1DM.

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PATIENT SATISFACTION WITH INTENSIVE INSULIN THERAPY IN TYPE 2 DIABETES: A RANDOMIZED TRIAL OF INSULIN PEN VS. PUMP

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Background and Aims: To better understand why intensive insulin therapy for persons with type 2 diabetes is difficult to initiate and sustain, we studied 126 insulin-treated type 2 patients randomized to multiple subcutaneous insulin (pen) injections (PEN; n=60) or continuous subcutaneous insulin (pump) infusion (PUMP; n=66). **Materials and Methods:** Patients were 61% male, 73% married, 77% Caucasian, aged 56±9 yrs, and had HbA_{1c} =8.1±1.2%, FPG=200±65mg/dL and BMI=32±5 kg/m². Satisfaction questionnaires were completed at baseline and at 16 and 24 weeks after randomization. **Results:** Mean±SE overall satisfaction (scaled 0 to 100) from baseline to endpoint improved more for PUMP (59.4±2.1 to 79.2±1.8) than for PEN (63.6±1.9 to 70.3±2.3), p<0.0001. Changes±SE from baseline to endpoint for the following satisfaction subscales (0 to 100) similarly showed more favorable effects for PUMP vs PEN: burden (16.3±2.1 vs 5.2±2.2), convenience (21.2±2.7 vs 5.5±2.4), efficacy (31.1±3.0 vs 14.1±3.3), flexibility (23.0±2.7 vs 6.3±2.4), general satisfaction (28.1±3.0 vs 10.2±3.6), and life interference (23.0±2.8 vs 5.1±2.6), all p<0.0001; hassle (16.2±2.7 vs 6.3±2.8), advocacy (25.6±3.4 vs 13.0±3.9), preference (31.8±4.2 vs 11.2±4.6), and social limitations (5.2±1.7 vs -1.0±1.7), all p<0.02. Mean decrease ±SE in endpoint FPG was significantly greater for PUMP vs PEN (-46±9 vs -12±12 mg/dL; p<0.02), but the decrease in HbA_{1c} (-0.62±0.14% vs -0.46±0.13%) was not. Over 90% of PUMP patients preferred the pump to their previous injectable regimen on each of 13 factors including dose adjustment (choosing pump = 97%), convenience when traveling (97%), ease of use, better glucose control, flexibility in daily activities and convenience (all 95%), overall preference and feeling better about themselves (both 93%). **Conclusions:** Type 2 patients demonstrated greater satisfaction with the pump compared to multiple daily injections using the pen. Increasing patient acceptance may improve compliance and facilitate the initiation and sustainability of intensive insulin therapy in type 2 diabetes.

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Continuous Subcutaneous Insulin Infusion versus Multiple Daily Injections in Children with Type 1 Diabetes

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Background and Aims: To compare the efficacy and feasibility of continuous subcutaneous insulin infusion (CSII) and multiple daily insulin injections (MDI) in children with type 1 diabetes.

Materials and Methods: The study sample included 13 children (6 males) aged 9.4 to 13.3 years with c-peptide-negative type 1 diabetes. Duration of disease was 2.8 to 11 years. An open randomized crossover design was used to compare 3.5 months of CSII to 3.5 months of MDI therapy for the following variables: diabetic control, incidence of symptomatic hypoglycemia and hyperglycemia, ketonuria, daily insulin requirement, diabetic ketoacidosis (DKA), body mass index standard deviation scores (BMI SDS) and safety. All comparisons were analyzed by ANOVA with repeated measures.

Results: HbA_{1c} values at the start and end of each treatment arm were similar (7.85–0.68 and 7.96–0.66 for the pump phase, 7.92–0.46 and 7.81–0.87 for the MDI phase). There were no differences between the treatment modes in frequency of mild symptomatic hypoglycemic or hyperglycemic events, both diurnal and nocturnal. There was one event of severe nocturnal hypoglycemia without coma in the MDI phase. Ketonuria tended to be more frequent during the pump therapy period (p=0.06), but there was no DKA. There was no significant change in BMI SDS during the entire study. Mean insulin dose at the end of the pump phase was lower than at the end of the MDI phase (0.89–0.14 and 1.09–0.21 u/kg/day, respectively, p<0.01). At the end of the study, 10/13 patients chose to continue pump therapy.

Conclusions: Diabetic control is similar in children treated with the insulin pump or with MDI, and it is comparable to that achieved in the Diabetes Control and Complications Trial (DCCT) by the intensive adolescent group. This study suggests that intensive insulin therapy by either CSII or MDI can be used safely in children and young adolescents with type 1 diabetes. In our series, most of the children preferred to continue with the insulin pump.

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Insulin pump therapy versus multiple daily injections in obese type 2 diabetic patients.

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Background and aims: Obese type 2 diabetic patients with severe insulin resistance tend to develop chronic hyperglycemia despite maximal treatment with diet, physical exercise and oral hypoglycemic agents. High dose insulin therapy in these patients does not usually lead to satisfactory glucose control and causes weight increase, which in turn aggravates insulin resistance and exacerbates other cardiovascular risk factors. A pilot study by the Israeli Diabetes Research Group showed that continuous insulin delivery by means of an insulin pump (Minimed 507R) was superior to multiple daily insulin injections in terms of glucose control, weight gain and total daily insulin requirement. **Material and methods:** 39 obese (BMI > 30) type 2 diabetic patients, not controlled (HbA_{1c} > 8.5%) on high dose insulin therapy (daily insulin requirement superior to 1 unit/kg body weight/day) were randomized to receive an 18-week insulin pump treatment followed (P-M group) or preceded (M-P group) by an 18-week multiple injection treatment, with a 12-week washout period in-between. **Results:** During the first 18 weeks: HbA_{1c} decreased from 10.2 ± 1.22 % (mean ± SD) at week 0 to 8.06 ± 1.15 % at week 18 in the M-P group (p = 0.001) and from 10.23 ± 1.43 to 7.65 ± 1.04 in the P-M group (p = 0.001). Daily insulin requirement decreased from 102 ± 24 units/day at week 0 to 85 ± 29 at week 18 (p=0.004) in the P-M group, and increased, though not significantly, from 113 ± 28 at week 0 to 116 ± 30 at week 18 in the M-P group. The absolute number of extreme hyperglycemic capillary blood glucose values decreased in the P-M group, but not significantly. There was no significant weight increase in either group. **Conclusions:** We conclude that obese type 2 patients on high dose insulin therapy can dramatically improve their blood glucose control without gaining weight either by intensifying the daily multiple injection treatment or by using an insulin pump, the latter allowing for reduction of daily insulin requirement. Further results (including continuous subcutaneous glucose monitoring) are expected to precise the individual benefit of insulin pump therapy versus multiple daily injections in such patients.

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Remission patterns after short-term continuous subcutaneous insulin infusion therapy in Korean type 2 diabetic patients.

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Background and Aims: Studies have found that chronic hyperglycemia can deteriorate pancreatic beta cell function and insulin sensitivity, and the normalization of blood glucose can reverse these functions. The purpose of the study is to evaluate the normoglycemic remission pattern after a certain period of continuous subcutaneous insulin infusion (CSII) therapy in Korean type 2 diabetic patients and to determine the clinical characteristics for remission.

Materials and Methods: Ninety-one mild type 2 diabetic patients were treated by CSII therapy via insulin pump (Sooil, Seoul, Korea) until they did not need any medication for glycemic control. Follow-up examinations took place once every month at an outpatient clinic for non-remitted patients and by phone for remitted patients, and their blood glucose levels and insulin dosage were monitored for fifteen to seventeen months.

Results: Overall, in 34.4 % of all subjects, remission was induced after 53.6±38.9 days of CSII therapy and lasted for an average of 13.6±8.9 months during the study periods. Blood glucose levels continuously decreased to be normalized at 7.3±1.2 days with maximum dosage of daily total insulin after CSII therapy. Total insulin dosage to normalize blood glucose levels gradually decreased until 14.4±2.7 days of CSII therapy in all subjects. Total daily insulin dosage did not significantly decrease any more from about 14 days of the therapy in non-remitted patients, but it did continuously decrease and reached at zero in remitted patients. In correlation analysis, remission was more frequently induced when patients started CSII therapy in younger age (r=-0.41, p<0.01), with higher body mass index (r=0.36, p<0.05), shorter diabetic duration (r=-0.48, p<0.01), lower post-prandial blood glucose levels (r=-0.51, p<0.01), higher post-prandial serum c-peptide levels (r=0.42, p<0.01), and less chronic diabetic complications (r=-0.47, p<0.01).

Conclusions: These findings suggest that long-term CSII therapy can induce remission in a significant proportion of Korean type 2 diabetic patients with mild symptoms. Thus, it is desirable that intensive insulin treatment by CSII be considered as not the last treatment, but an initial management of mild type 2 diabetic patients.

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Thiazolidinedione Action

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CHARACTERISATION OF METABOLIC INTERMEDIATES AND FLUXES IN ROSIGLITAZONE-TREATED ZUCKER FATTY RATS: AN *IN VIVO* NUCLEAR MAGNETIC RESONANCE STUDY

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Background and Aims: Rosiglitazone (RSG), a potent insulin sensitiser, was administered to Zucker fatty rats. *In vivo* ^{13}C nuclear magnetic resonance (NMR) spectroscopy was used to measure skeletal muscle glucose uptake and its distributed fluxes (glycogen synthesis and glycolysis) and ^{31}P NMR was used to measure simultaneous changes in glucose-6-phosphate (G6P) during a euglycaemic-hyperinsulinaemic clamp. ^1H NMR was used to measure intramyocellular lipid content. The aim of the study was to non-invasively measure metabolic intermediates and fluxes to provide insight into the therapeutic mechanism of action of RSG. **Materials and Methods:** Three groups of rats (fatty-RSG, FR; fatty-vehicle, FV; lean-vehicle, LV) weighing ~300 g were chronically catheterised prior to a 7-day treatment regimen (3 mg/kg RSG or vehicle via oral gavage). Rates of glycolysis and glycogen synthesis were assessed after treatment by monitoring $1,6\text{-}^{13}\text{C}$ glucose label incorporation into $1\text{-}^{13}\text{C}$ glycogen, $3\text{-}^{13}\text{C}$ lactate and $3\text{-}^{13}\text{C}$ alanine during a euglycaemic ($\sim 6\text{-}8\text{ mmol/l}$)-hyperinsulinaemic (10 mU/kg/min) clamp in the hindlimb of conscious rats. **Results:** The FR group exhibited a significant increase in insulin sensitivity, reflected by an increased whole-body glucose disposal rate during the clamp (24.4 ± 1.9 vs 17.6 ± 1.4 and 33.2 ± 2.0 mg/kg/min in FR vs FV [$p < 0.05$] and LV groups [$p < 0.01$], respectively). The increased insulin-stimulated glucose disposal in the FR group was associated with a normalisation of the glycolytic flux (52.9 ± 1.1 to LV (56.2 ± 16.6) vs FV (18.8 ± 8.6 nmol/g/min, $p < 0.02$) and glycogen synthesis flux (56.3 ± 11.5 to LV (75.2 ± 15.3) vs FV (16.6 ± 12.8 nmol/g/min, $p < 0.05$). [G6P] increased in the FR and LV groups vs baseline during the clamp ($+13.0 \pm 11.1$ and $+16.9 \pm 5.8\%$ respectively), whereas the FV group decreased ($-23.3 \pm 13.4\%$, $p < 0.05$). There were no differences between groups in intramyocellular glucose as measured by biochemical assay. The intra- to extramyocellular lipid ratio, measured in a separate group of rats, decreased from 0.43 ± 0.12 at baseline to 0.04 ± 0.01 after 7 days' treatment with RSG ($p < 0.01$). **Conclusions:** These data suggest that the increased insulin-stimulated glucose disposal associated with RSG treatment in muscle can be attributed to a normalisation of glucose transport function and may be in part attributable to decreased intramyocellular lipid deposition.

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Ras and Ral-dependent phosphorylation of ATF2 mediates activation of the c-jun promoter by insulin

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Background and Aims: cJun is an essential component of the transcription factor AP-1, and plays a critical role in development, cell proliferation and differentiation, for instance of adipocytes. Insulin rapidly induces the expression of the c-jun gene. Here, we have examined the signalling pathways regulating c-jun expression. **Materials and Methods:** Studies were performed in A14 cells, which are NIH3T3 fibroblasts overexpressing the human insulin receptor. **Results:** Insulin induces the expression of the c-jun gene by enhancing the activity of the transcription factor ATF2 on the c-jun promoter. Insulin transactivates ATF2 via phosphorylation on its proline-directed sites Thr69 and Thr71. Insulin was found to activate ATF2 via different signal transduction pathways than inducers of cellular stress. Stress-, but not insulin-, induced activation of ATF2 can be inhibited by dominant-negative mutants and/or inhibitors of the stress-inducible signalling enzymes Rac and SEK. In contrast, insulin-induced phosphorylation of ATF2 Thr69+71 could only be inhibited by dominant-negative versions of Ras and its effector Ral, which on their turn, have no effect on the activation of ATF2 by cellular stress. In line with this, phosphorylation of ATF2 and induction of ATF2-dependent c-jun transcription in the absence of insulin was efficiently induced by ectopic expression of a constitutively active mutant of Ras and by active version of the Ras effector enzyme Rlf, an exchange factor for the small GTP-binding protein Ral. The kinases phosphorylating ATF2 in response to insulin are so far unknown. Insulin only very inefficiently activates the known ATF2 Thr69+71 kinases p38 and JNK. When analysing extracts of insulin-treated cells for ATF2 kinase activity using ion column chromatography, at least two independent ATF2 kinase activities were identified: a strongly inducible Thr71 kinase activity (colocalizing with ERK), and a very weakly induced Thr69+71 kinase activity (colocalizing with p38). Interestingly, the activation of the ATF2-Thr71 kinase could be abrogated by inhibitors of Ras and MEK, whereas activation of the ATF2 Thr69+71 kinase was prevented by inhibitors of Ras, Ral, and p38. This leads to the possibility that two distinct ATF2 kinase activities, activated by different signalling pathways, need to cooperate to induce the activation of ATF2 by insulin *in vivo*. **Conclusions:** 1. Insulin stimulates c-jun expression via the Ras and Ral-dependent phosphorylation of ATF2 on Thr69 and 71. 2. Phosphorylation of ATF2 is a multi-step process; Thr71 phosphorylation is induced by ERK, and Thr69 phosphorylation by p38.

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NITRIC OXIDE SYNTHASE INHIBITION DECREASES THE INCREASED INSULIN ACTION BY PIOGLITAZONE IN THE FRUCTOSE-FED RAT

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Background and Aims: Recent studies have demonstrated that Pioglitazone (PIO) has the capacity to ameliorate insulin resistance. However, the precise mechanism of this agent remains unknown. Our preliminary study shows that not only PIO but also a nitric oxide (NO) donor, sodium nitroprusside, can improve insulin resistance induced by high-fructose feeding. The purpose of this study was to investigate whether NO synthase inhibition influences the increased insulin action by PIO in fructose-fed rats.

Materials and Methods: Twenty-four male Wistar rats aged 6wk were randomly divided into three groups; a standard chow-diet (n=6), a high-fructose diet (3wk, n=6) and a high fructose diet plus PIO (3wk, n=12). *In vivo* insulin action was determined by the sequential euglycemic clamp technique at two different insulin infusion rates (3 and 30 mU/kg/min) under the awake condition. A NO synthase inhibitor, NG-monomethyl-L-arginine (L-NMMA, 1mg/kg/min), was infused during the sequential euglycemic clamp procedure in half of "high-fructose diet plus PIO" rats. Glucose disposal rate (GDR) was regarded as an index of whole body insulin action.

Results: Plasma insulin concentrations during the 3- and 30- mU/kg/min insulin infusions were 30-40 and 600-800 $\mu\text{U/ml}$, respectively and blood glucose was clamped at a fasting level by adjustment of i. v. glucose infusion. At the 3- mU/kg/min insulin infusion, high-fructose feeding produced a marked decrease in GDR compared with the chow-fed rats (5.5 ± 0.1 vs 9.5 ± 0.2 mg/kg/min, $p < 0.05$). PIO treatment showed a significant increase in GDR (7.9 ± 0.6 mg/kg/min, $p < 0.05$) and reached similar levels as the chow-fed rats. However, increased GDRs were decreased by L-NMMA administration (5.3 ± 0.7 mg/kg/min). At the 30- mU/kg/min insulin infusion, the same tendency as the 3- mU/kg/min insulin infusion was found. GDRs in fructose-fed rats given PIO were significantly greater than those in fructose-fed rats without PIO (40.1 ± 1.2 vs 30.1 ± 0.5 mg/kg/min, $p < 0.05$). Delta increase in GDR by PIO was disappeared by L-NMMA infusion (32.2 ± 1.4 mg/kg/min).

Conclusions: These results suggest that NO synthase inhibition can decrease the improved insulin resistance by PIO in fructose-fed rats. Therefore, it could be concluded that NO plays an important role in the improvement of insulin action by PIO.

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INSULIN SENSITIVITY AND INTRAMYOCYELLULAR LIPIDS IN ROSIGLITAZONE TREATED ZUCKER FATTY RATS

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Background: Peripheral insulin sensitivity depends predominantly on the glucose utilisation of muscle tissue. There is growing evidence that increased levels of lipids in muscle cells are involved in insulin resistance. Thiazolidinediones are ligands for PPAR γ transcription factors, which are involved in fat cell differentiation. Thiazolidinediones induce a permanent remodelling of adipose tissue and increase peripheral insulin sensitivity in insulin resistant animals. Aim of the study was to investigate the improvement of insulin sensitivity in rosiglitazone pretreated rats and its effect on intramyocellular lipid content.

Material and Methods: Insulin resistant Zucker Fatty rats (fa/fa) were pretreated with rosiglitazone, 7 mg/kg orally for 3 weeks. Insulin sensitivity was measured by the euglycemic-hyperinsulinemic glucose clamp technique (4.8 and 9.6 mU/kg/min). At the end of the clamp study the soleus muscle was isolated for electron microscopic evaluation of intramyocellular lipid droplets.

Results: Pretreatment with rosiglitazone caused a marked improvement of whole body glucose utilisation. Glucose infusion rate in control rats was for the low and high insulin infusion rate 1.8 and 5.6 mg/kg/min, respectively. In treated rats glucose infusion rate increased for the low and high insulin infusion rate to 13 and 15 mg/kg/min, respectively. The transmission electron microscopic examination revealed that the soleus muscles of rosiglitazone-treated rats were nearly void of intramyocellular lipid droplets in contrast to the muscles of the control group.

Conclusions: It is concluded that the improvement of insulin-sensitivity by rosiglitazone is related to its effect on lipid metabolism. It is hypothesized that insulin-sensitivity, which is predominantly a function of the muscle tissue during the clamp study, is improved indirectly by the ability of rosiglitazone to redirect lipid storage back to the fat tissue.

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ROSIGLITAZONE PROTECTS THE DIABETIC HEART FROM ISCHAEMIA-REPERFUSION INJURY: ROLE OF THE JNK/AP-1 PATHWAY

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Background and Aims: The risk of ischaemic heart disease is greatly elevated, and the survival rate following a myocardial infarction is markedly diminished, in patients with Type 2 diabetes mellitus, relative to the non-diabetic population. Rosiglitazone (RSG), a new insulin-sensitising agent with potent agonist activity at the PPAR γ nuclear receptor, has been studied here for its ability to protect against ischaemic/reperfusion injury.

Materials and Methods: Experiments were conducted in isolated working hearts from normal Wistar rats and from rats treated 4 weeks earlier with streptozotocin (55 mg/kg i. v.), to induce insulinopenic diabetes. After a 30 min control period, hearts were subjected to 30 min of normothermic, zero-flow ischaemia and then reperfused normally for a final 30 min.

Results: RSG (1 μ M), added to the perfusate 15 min before ischaemia had no effect on baseline cardiac function, but markedly improved indices of cardiac function during post-ischaemic reperfusion. For example, in hearts from normoglycaemic rats, post-ischaemic recoveries in aortic flow after 5 min of reperfusion were (mean \pm SEM) 0 and 11.6 \pm 4.0 ml/min in control and RSG-treated groups (p <0.05), respectively. In hearts from diabetic rats, the corresponding aortic flow values were 8.5 \pm 3.4 and 26.8 \pm 3.3 ml/min (p <0.05). Qualitatively similar cardioprotective effects were seen *ex vivo*, after chronic oral pre-treatment with RSG (10 μ mol/kg/day for 4 weeks). Further, using western immunoblotting, we demonstrated that PPAR γ was present in rat hearts and that RSG inhibited Jun N-terminal kinase (JNK) phosphorylation in hearts from normoglycaemic or diabetic rats, following ischaemic-reperfusion injury. Consistent with this finding was the additional observation that RSG inhibited AP-1 DNA binding.

Conclusions: These findings suggest that the cardioprotective action of RSG involves preventing excitation of a stress-activated protein kinase pathway. The data raise the possibility that, in diabetic patients, RSG may reduce the risk of cardiac injury following an ischaemic event.

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INSULIN SENSITISATION BY ROSIGLITAZONE: EFFECTS ON METABOLISM AND GENE EXPRESSION

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Background and Aims: The literature is fragmented on the changes in gene expression evoked by thiazolidinediones in relation to improved insulin action. We studied the effects of rosiglitazone (RSG) in a rat model of dietary obesity. **Materials and Methods:** RSG (3 mg/kg/day p. o. for 14 days) was given to male Wistar rats fed a highly palatable diet, either *ad libitum* (RSG-AL), or restricted (RSG-R) to that of vehicle-treated rats (CON) to negate the effect of RSG-related weight gain. A further group received normal chow *ad libitum* (CHOW). Plasma constituents and gene transcripts (TaqMan analysis) in epididymal white adipose tissue (WAT) and gastrocnemius muscle (GM) were measured. **Results:** Relative to CHOW, CON rats were hyperinsulinaemic (118 \pm 11 vs 85 \pm 2 pmol/l, p <0.05) and hypertriglyceridaemic (0.48 \pm 0.04 vs 0.26 \pm 0.05 mmol/l, p <0.01). RSG reduced plasma insulin (68 \pm 6, 84 \pm 3 pmol/l for RSG-R, RSG-AL; p <0.01 vs CON), triglycerides (0.13 \pm 0.01, 0.18 \pm 0.01 vs 0.48 \pm 0.04 mmol/l, p <0.0001) and free fatty acids (0.45 \pm 0.04, 0.47 \pm 0.03 vs 0.87 \pm 0.06 mmol/l, p <0.0001). Improved insulin action and plasma lipids by RSG coincided with significant (p <0.05) changes in mRNA levels of several genes relative to CON. In WAT of RSG-R and RSG-AL, fatty acid synthase (FAS) rose 2.86 and 2.35-fold, respectively, and fatty acid binding protein 3 (FABP3) by 29.3 and 25.3-fold. GLUT-4 and FABP4 increased in RSG-R (by 1.71 and 1.52-fold). Resistin (by ~60%), leptin (~50%) and TNF- α (39%) mRNAs were reduced by RSG, though TNF- α only in RSG-R. Transcripts that increased significantly in GM were hormone-sensitive lipase (1.44, 1.51-fold in RSG-R, RSG-AL), FAS (2.84, 4.98-fold) and FABP4 (2.37, 2.90-fold), though we cannot exclude the possibility that these were of adipocyte origin. **Conclusion:** Our data suggest that RSG-related enhanced insulin action may be associated with changes in both muscle and fat gene expression.

OP 18

Metabolic Changes in Pregnancy

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THE ROLE OF GLYCOPROTEIN PC-1 IN INSULIN SIGNALING PATHWAY IN ADIPOCYTES FROM TYPE 2 DIABETIC PREGNANT WOMEN AND WOMEN WITH GESTATIONAL DIABETES

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Background and Aims: The cellular mechanisms for the insulin resistance of pregnancy and gestational diabetes mellitus (GDM) are unknown. The membrane protein plasma cell membrane glycoprotein (PC-1) has been identified as an inhibitor of insulin receptor tyrosine kinase activity (IRTK) and may have a role in insulin resistance. The aim of the study was to examine the effects on glucose transport and changes in PC-1, IRTK and IRS-1.

Materials and Methods: The isolated omental adipocytes were obtained during elective cesarean sections from three groups of subjects: Type 2 diabetic pregnant women (n=6), women with GDM (n=10) and pregnant women with normal glucose tolerance (n=6) as control subjects. **Results:** Insulin stimulated glucose transport was reduced 38% in GDM and 60% in Type 2 diabetic gravidas, compared to the controls. After maximal insulin stimulation of adipocytes IRTK phosphorylation was decreased 25% in GDM and 40% in Type 2 diabetic gravidas, compared to the controls. We also found that IRS-1 phosphorylation was decreased by 30% and 55%, respectively. On the other hand, PC-1 content in adipocytes from GDM was increased 60% and 95% in Type 2 diabetic gravidas, compared to the pregnant control subjects.

Conclusions: Our results indicate that GDM and Type 2 diabetic gravidas have increased PC-1 content and suggest that this may contribute to decrease phosphorylation levels of IRTK and IRS-1. Furthermore, these postreceptor defects in insulin signaling pathway worsen in both groups compared to the normal pregnancy, however results from Type 2 diabetic gravidas show that pre-existing insulin resistance worsen the signaling pathway even more.

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IMPACT OF INSULIN SENSITIVITY ON BODY COMPOSITION, PLASMA LEPTIN, LIPIDS AND PAI-1 CONCENTRATIONS IN WOMEN WITH FORMER GESTATIONAL DIABETES

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Background and aims: Women with prior gestational diabetes (GDM) are at high risk for later Type 2 Diabetes and might thus share parameters of the metabolic syndrome. Therefore we intended to study body composition, plasma leptin and parameters of the lipid and fibrinolytic system in relation to insulin sensitivity in GDMs. **Methods:** 51 women (Age: 33.5 \pm 4.6 years, body mass index (BMI): 27.5 \pm 5.6 kg/m², basal glucose: 91.7 \pm 10.9 mg/100ml, insulin: 10.4 \pm 8.3 μ U/ml and C-peptide: 208.9 \pm 104.6 ng/100ml; mean \pm SD) were studied 12-16 weeks postpartum. The insulin sensitivity index [S_i : 10⁻⁴ min⁻¹ (μ U/ml)⁻¹] and the disposition index [DI: S_i x acute insulin response to glucose (pmol/l)] were derived from insulin modified frequently sampled glucose tolerance tests and the insulin sensitivity parameter OGIS (ml min⁻¹ m⁻²) from OGTTs by mathematical model analysis. Insulin resistance was defined as S_i < 3. Body composition was measured by bioelectrical impedance (BIA), plasma lipids by gel electrophoresis, PAI-1 by ELISA, TNF- α and plasma leptin by RIA analysis. **Results:** 24 women were insulin-resistant (IR: S_i : 1.84 \pm 0.8 and OGIS: 370.5 \pm 27.6) compared to 27 women with normal insulin sensitivity (IS: S_i : 5.53 \pm 2.2 and OGIS: 460.2 \pm 57.3; p <0.0001, ANOVA). BMI (29.7 \pm 5.9 vs. 24.9 \pm 3.5 kg/m², p <0.001), fat-mass in percent of body weight (FM: 30.5 \pm 10.9 vs. 20.8 \pm 7.7, p <0.01), WHR (0.86 \pm 0.05 vs. 0.81 \pm 0.06, p <0.05), plasma leptin (18.4 \pm 6.7 vs. 12.8 \pm 6.7 ng/ml, p <0.01) and PAI-1 (36.8 \pm 21.0 vs. 19.3 \pm 12.4 ng/ml, p <0.001) levels were higher, while BCM percent of body weight (BCM) 35.7 \pm 4.0 vs. 38.7 \pm 4.3, p <0.05), HDL cholesterol (50.3 \pm 14.1 vs. 60 \pm 13.0 mg/dl, p <0.05) and DI (0.08 \pm 0.006 vs. 0.15 \pm 0.07, p <0.001) were lower in IR. S_i related to parameters of body composition (BMI r^2 =0.52, p <0.0001; FM r^2 =0.59, p <0.0001; BCM r^2 =0.52, p <0.0001) and independent of body fat to plasma leptin (r^2 =0.41, p <0.001) and PAI-1 (r^2 =0.61, p <0.0001), but was not associated with TNF- α . **Conclusion:** About 50% of GDMs studied were still markedly insulin-resistant 12-16 weeks postpartum. This subgroup also had a relative beta-cell secretion defect and was furthermore characterized by higher fat mass, plasma leptin and PAI-1 concentrations and changes in lipid profile as commonly found in diabetic patients.

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INCREASED FAT MASS, REDUCED $\dot{V}O_2$ MAX AND FEATURES OF THE METABOLIC SYNDROME IN HEALTHY LEAN WOMEN WITH PREVIOUS GESTATIONAL DIABETES MELLITUS.

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Background and aims: Healthy women with previous GDM have a considerably increased risk for later development of type 2 diabetes. The aim of the present study was to further characterize body composition, $\dot{V}O_2$ max and aspects of glucose and lipid metabolism in these healthy but potentially prediabetic individuals. **Materials and Methods:** We examined 24 lean and anti-GAD negative women with previous GDM and 19 women with a normal pregnancy. Body composition was determined by means of bioelectric impedance and $\dot{V}O_2$ max by a bicycle ergometer test. Glucose and lipid homeostasis were characterized during OGTT and IVGTT. Insulin sensitivity was estimated as the glucose disappearance constant (K_{it}) during IVGTT. **Results:** Post-GDM women and controls were comparable with respect to age, BMI and total body weight, however % body fat (25.9 ± 1.0 vs 22.3 ± 1.5 %) was significantly increased and $\dot{V}O_2$ max was significantly reduced (33.8 ± 1.5 vs 38.2 ± 1.5 ml/kg/min) in post-GDM women ($p(\text{both}) < 0.05$). All subjects had a normal OGTT, but AUC of blood glucose (305 ± 23 vs 239 ± 19 mmol/l/min, $p < 0.01$) was markedly increased in the post-GDM group. Similar findings were obtained with respect to serum insulin and C-peptide ($p(\text{both}) < 0.01$). In the fasting state post-GDM women had hypertriglyceridemia (1.14 ± 0.10 vs 0.85 ± 0.07 mM), increased blood glycerol (55.1 ± 3.9 vs 38.3 ± 3.6 μ M) and an increased total cholesterol/HDL-cholesterol ratio (4.2 ± 0.3 vs 3.5 ± 0.2) ($p(\text{all}) < 0.05$). Moreover, the insulin induced suppression of FFA two hours following OGTT was attenuated (0.09 ± 0.01 vs 0.05 ± 0.01 mM, $p < 0.05$) in post-GDM subjects. K_{it} was diminished in the post-GDM group (1.30 ± 0.12 vs 1.54 ± 0.12 , $p = 0.08$) whereas first-phase serum insulin was comparable in the two groups (1486 ± 170 vs 1512 ± 160 pM/min). **Conclusion:** In conclusion the present study demonstrates that healthy, lean women with prior GDM are characterized by an increased relative fat mass and reduced $\dot{V}O_2$ max compared to controls with similar BMI. Despite a normal OGTT, the post-GDM women display several features of the metabolic syndrome. Insulin secretion was normal or exaggerated but appears inappropriately low considering the degree of insulin resistance.

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TISSUE SPECIFIC EXPRESSION OF ACETYL-CoA CARBOXYLASE, FATTY ACID SYNTHASE AND CARNITINE PALMITOYL TRANSFERASE 1 IN WEANLING RATS EXPOSED TO A LOW PROTEIN DIET IN UTERO

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Background and Aims: Low birth weight and poor growth during childhood has been associated with early-onset abdominal obesity, type 2 diabetes, and hypertension in adult life (The Barker Hypothesis). Maternal protein energy malnutrition (PEM) in rats results in low birth weight and reduced growth after birth, but increased adiposity in later life. We have previously shown that maternal PEM in rats results in changes in hepatic gene expression in weanling rats which could result in increased fat deposition, ie increased expression of acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS), enzymes involved in fat synthesis as well as decreased expression of carnitine palmitoyl transferase 1 (CPT-1), a key enzyme for fatty acid oxidation. The aim of this study was to investigate the changes in the expression levels of these 3 genes in quadriceps muscle taken from the same animals.

Materials and Methods: Pregnant mothers were placed on one of two isocaloric diets (a low protein diet (LP, 8% protein) or a control diet (CONT, 20% protein)) the day after pregnancy was confirmed. Mothers remained on the diet throughout pregnancy and lactation. Pups were weaned on day 25 after delivery and sacrificed on the morning of day 26. Quadriceps muscle was removed for analysis of ACC, FAS and CPT-1 mRNA expression using semi-quantitative reverse transcription polymerase chain reaction. The housekeeping gene b-actin was used as an internal control.

Results: We have previously shown that the LP weanlings had a 40% lower body weight when sacrificed as well as a 4-fold increase in expression of ACC and FAS and a 50% decrease in CPT-1 in the liver. In muscle however, there were no significant differences in the expression of these 3 enzymes relative to β actin; Cont vs LP: ACC: 0.89 ± 0.08 vs 0.72 ± 0.08 ; FAS: 0.61 ± 0.07 vs 0.64 ± 0.1 ; CPT-1: 0.91 ± 0.13 vs 0.94 ± 0.16 . These similarities in expression occurred despite increased plasma glucose (9.1 ± 0.5 vs 11.2 ± 0.5 mM), triglycerides (1.41 ± 0.2 vs 1.99 ± 0.2 mM) and reduced plasma free fatty acids (0.432 ± 0.04 vs 0.087 ± 0.03 mM) and insulin (1.48 ± 0.3 vs 0.88 ± 0.1 ng/ml), all of which are known to regulate the expression of these genes.

Conclusions: Whereas the gene expression pattern of ACC, FAS and CPT-1 in the liver of LP animals was found to be conducive to fat partitioning, muscle expression was unaltered showing that maternal PEM in utero and during lactation alters gene expression in a tissue specific manner.

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THE EFFECT OF MATERNAL PROTEIN RESTRICTION ON HEPATIC GLYCOGEN METABOLISM IN WEANLING RATS

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Background and Aims: Poor in utero nutrition can result in reduced fetal growth which is associated with insulin resistance in later life. The development of insulin resistance may be due to alterations in gene expression in enzymes involved in carbohydrate and lipid metabolism. We have previously found that weanlings from mothers fed low protein diets throughout gestation and lactation have increased plasma glucose and decreased plasma insulin concentrations compared to weanlings from mothers fed control diets. This study aimed to determine whether maternal protein restriction alters the expression and/or the activity of hepatic enzymes involved in glycogen metabolism in weanling rats. **Materials and Methods:** Mated females were placed on either a control (20% protein) (Con) or low protein (8% protein) (LP) isocaloric diet. Offspring were killed (fed) at 26 days, tissues and blood were collected. Liver glycogen content, glycogen phosphorylase (GP) and glycogen synthase (GS) gene expression and activities were measured. **Results:** There were no differences in the expression of liver GP (LP: 0.856 ± 0.26 , Con: 0.676 ± 0.11 , $p = 0.4$) and GS (LP: 1.366 ± 0.25 , Con: 1.167 ± 0.12 , $p = 0.4$) genes relative to β actin. The LP offspring had greater active GP activity (nmol GlP/min/ng wet weight) (LP: 7.15 ± 0.55 , Con: 5.28 ± 0.52 , $p = 0.02$). Total GS activity (nmol UDPG/min/ng wet weight) was reduced in LP offspring (LP: 0.226 ± 0.023 , Con: 0.307 ± 0.026 , $p < 0.001$) as was active GS activity (LP: 0.158 ± 0.013 , Con: 0.218 ± 0.023 , $p = 0.002$). LP offspring had a 5-fold increase in liver glycogen content (μ mol glucose/g wet weight). (LP: 336.9 ± 86.99 , Con: 67.3 ± 15.86 $p < 0.0001$). **Conclusions:** In utero malnutrition had no effect at weaning on the expression of key enzymes involved in hepatic glycogen synthesis and degradation but did have an effect on the activities of these enzymes. The changes seen in the activities of these enzymes towards decreased glycogen synthesis and increased glycogenolysis are consistent with the reduction in plasma insulin and raised plasma glucose previously found and suggest that these pathways in the livers of LP weanlings remain insulin sensitive. However, the changes in the activities of these enzymes are inconsistent with the elevated hepatic glycogen content found after LP feeding, suggesting dysregulation of other pathways which contribute to glycogen formation.

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The influence of maternal thinness and age in twin pregnancies on insulin resistance in the offspring

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Background and Aims: There is strong evidence that low birthweight is associated with glucose intolerance and diabetes. However, it is not clear whether fetal or maternal factors are responsible for this association. We have carried out a twin study to determine the relative influence of fetoplacental factors (as evidenced by associations between birthweight and outcomes in twin pairs who were growth discordant at birth) or maternal influences such as body composition.

Materials and Methods: We identified a sample of 423 twin pairs (250 MZ and 173 DZ) from the East Flanders Prospective Twin Survey who were born between 1964 and 1982. Data collected in this study included the mother's body composition and weight gain during pregnancy, the twins' birthweights and gestational age. The twins (aged 18-34 years) attended a research centre for measurement of height, weight, waist-to-hip ratio, and fasting insulin and glucose concentrations.

Results: We found little evidence that among discordant twin pairs the lighter twin had abnormal glucose-insulin metabolism in the adult life. However, both a low maternal body mass index and older maternal age at delivery were associated with hyperinsulinaemia and evidence of insulin resistance in the offspring. Fasting insulin increased by 1.3% [95CI 0.1-2.6%] per unit fall in maternal BMI and by 1.1% [95CI 0.02-2.0%] per year increase in maternal age. These associations were independent of the twins' BMI and waist-to-hip ratio or their zygosity.

Conclusions: These novel findings suggest that in twin pregnancies maternal factors are more important than fetoplacental factors in determining glucose-insulin metabolism in the offspring.

OP 19 Beta-Cell Gene Expression

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The effect of chronic exposure to fatty acids on gene expression profile in clonal insulin-producing cells:

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Backgrounds and Aims. Changes of gene expression are involved in the mechanism of lipotoxicity in beta cells. Extensive investigation of gene expression profiles may help to characterise the mechanism. **Materials and Methods.** INS-1 cells were cultured with palmitate (0, 50 and 200 μ M) for up to 72 days. We studied changes in the insulin secretion capacity at day 2, 9, 16, 20, 37, 44, and 72, and 8740 gene expressions using high-density oligonucleotide microarray (gene chip) at day 2 and day 44. A subset of genes was verified by real-time RT-PCR. **Results.** 1) Increased basal insulin secretion at 1.0 mM glucose is more pronounced (97 ± 8 and 57 ± 4 ng/mg protein/60 min respectively at day 44 vs. 20 ± 1 in control group, $P < 0.05$) and appeared earlier (2 days vs. 5 weeks) in cells cultured at 200 μ M than at 50 μ M palmitate. 2) Glucose and fatty acid-stimulated insulin secretion was enhanced from day 2 to day 30 but declined after 37 days in cells cultured at 200 μ M palmitate. 3) There were 11 and 99 genes at day 2 and 134 and 159 genes that changed expression at day 44 after culture with 50 μ M and 200 μ M palmitate, respectively. 4) The results were good correlation between gene chip and those from RT-PCR; 5) changes in expression were found in genes involved in nutrient metabolism, signal transduction, ion channels, etc. Interestingly, genes involved in fatty acid oxidation were up-regulated in cells culture with 200 μ M palmitate whereas genes involved in glucose oxidation were down-regulated in cells exposed to longer time or higher concentration of palmitate. A combined suppression of insulin receptor, hepatic nuclear factor 4, cytokine-inducible src-homology-2 containing protein, glycogen phosphorylase and insulin receptor substrate-2 gene was found in cells cultured with 200 μ M palmitate at day 44. **Conclusion.** Palmitate could modify insulin secretion and gene expression of INS-1 cells. The alteration of gene expression was dose and time dependent to palmitate exposure. Rat's glucose-fatty acids cycle seems operative in mRNA levels in INS-1 cells that exposed to palmitate. A combined modification of expression of genes involved in different cell functions may contribute to the change of insulin secretion after exposure INS-1 cells to palmitate.

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The role of ICER in Pancreatic beta cell : Study in ICER transgenic mice

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Background and Aims: Previously, we have reported that the expression of ICER, a powerful transcriptional repressor of insulin gene, was increased in pancreatic islets of diabetic animal. To ascertain the specific role of ICER in pancreatic beta cell in vivo, we have now generated ICER transgenic mice.

Materials and Methods: The insulin mRNA level and the capacity of insulin secretion to glucose stimulation in ICER transgenic mice were examined by RT-PCR and batch incubation method, respectively. Serum parameters were determined by using ELISA kit. The morphological abnormalities in distribution and population of pancreatic islets cell were analyzed by immunohistochemistry.

Results: ICER transgenic mice displayed severe hyperglycemia, and failed to develop the body weight. The expression of insulin mRNA and the serum insulin concentration were remarkably reduced, whereas serum ketone and glucagon levels were elevated in ICER transgenic mice. Upon oral glucose challenge, ICER transgenic mice kept abnormally high glucose levels, and did not return to basal values during the study. Insulin secretion to glucose stimulation of the islets isolated from ICER transgenic mice was completely arrested. Furthermore, immunohistochemical studies revealed the deformity and reduction of beta cell and a significant increase in the number of alpha cell combined with the abnormal distribution within the islets in ICER transgenic mice.

Conclusions: ICER plays critical roles in regulating the insulin gene transcription and maintaining the beta cell population in a differential state.

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Effects of cytokine stimulation on the gene expression profile in insulin-producing RINm5F cells

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Background and Aims: Type 1 diabetes mellitus is an autoimmune disease, where T-cells and macrophages infiltrate the islets of Langerhans and lead to specific destruction of pancreatic beta-cells. Cytokines which are released by these infiltrating mononuclear cells are able to activate genes and proteins in the beta-cell which determine whether these cells will survive or undergo apoptosis or necrosis. To identify genes which are involved in the cytokine mediated beta-cell destruction a restriction fragment differential display technique was used in this study. **Materials and Methods:** Insulin-producing RINm5F tissue culture cells were exposed for 6 hours to a cytokine mix of IL-1-beta (60 U/ml), TNF-alpha (14 U/ml) and IFN-gamma (185 U/ml). The mRNA of the RINm5F cells was isolated and used for a restriction fragment differential display to obtain a comprehensive quantitative gene expression profile. The profile of cytokine stimulated RINm5F cells was compared to that of untreated control cells. Differentially expressed genes were quantified by the gel analysis software GelPro and identified by the Displayfit database.

Results: RINm5F cells exposed to the cytokine mix showed more than 50 differentially expressed genes in comparison with control cells. Apart from known cytokine regulated genes like inducible nitric oxide synthase (iNOS) and heat shock protein 70 (hsp70) several other differentially expressed genes grouped in ion channels, receptors, enzymes, regulatory and structural proteins were identified. Six different types of ion channels were down-regulated indicating an imbalance of ion homeostasis. In the group of regulatory proteins the expression of the calcineurin inhibitor gene was down-regulated resulting in an increase of iNOS activity and a stimulation of endocytosis. Further support for an interference of cytokines with the endocytosis pathway are observed expression increases of the megalin and clathrin genes.

Conclusions: With the restriction fragment differential display technique it was possible to identify several new differentially expressed genes under cytokine stimulation. The up-regulation of several genes of the endocytosis pathway is indicative for the activation of a cytoprotective pathway in the cells against cytokine-induced toxicity. This knowledge can provide a better understanding of the molecular mechanisms leading to beta-cell destruction during diabetes type 1 manifestation and open perspectives for new treatment strategies.

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The Protein Tyrosine Phosphatase like Molecule IA-2 as a Model for Paracrine Regulation by Insulin

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Background and Aims: IA-2 is a major autoantigen in Type 1 diabetes. It is localised on secretory granules of islets and neuroendocrine tissues and its tyrosine phosphatase like structure is highly conserved between species. Activity, function and precise regulation are unknown. IA-2 is undetectable in rat foetal development from day 13 onwards but up-regulated after birth. This study investigates the regulation of IA-2 in rat and human pancreatic islets.

Materials and Methods: Islets isolated from 1, 5 and 10 day old and adult rats as well as human islets were isolated and cultured for 1, 2 or 10 days in RPMI containing 3, 6, 10 or 20 mM glucose and supplemented with exogenous insulin (3, 16 and 160 nM), tolbutamide, forskolin, IBMX or diazoxide, respectively. IA-2 protein and RNA content of the islets was quantified by Western blotting and Real Time PCR, respectively, and compared to the insulin accumulated in the culture medium.

Results: IA-2 is up-regulated after birth in parallel with the maturation of insulin secretion. In vitro, IA-2 expression was strongly influenced by glucose. Culture in 10 mM compared to 6 mM glucose resulted within 1 day in an 7-fold (rat islets) or 4-fold (human islets) increase in IA-2 content. We have studied whether glucose regulates IA-2 expression directly, or indirectly via secreted insulin. In rat islets, addition of exogenous insulin, tolbutamide, forskolin or IBMX stimulated IA-2 expression by 4-, 2-, 2.5- or 3-fold, respectively, compared to culture in 6 mM glucose alone. When added to medium containing 10 mM glucose, none of these compounds resulted in a further increase of the IA-2 content. IA-2 expression at 10 mM glucose could be blocked when insulin secretion was inhibited using diazoxide. Similarly, in human islets, exogenous insulin and insulin secretagogues increased IA-2 content while diazoxide blocked the increase mediated by glucose. The up-regulation of IA-2 protein content was paralleled by an increase of IA-2 mRNA.

Conclusions: IA-2 expression is up-regulated by glucose, exogenous insulin and insulin secretagogues and blocked by inhibitors of insulin secretion. These results demonstrate that the stimulation of the IA-2 expression by glucose is mediated via insulin and regulated at the level of transcription. Insulin has been shown to feed back on insulin transcription but few is known about its influence on other beta cell specific proteins. This paracrine mechanism might account for the increase in IA-2 after birth and could be important for other aspects of islet maturation.

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Sterol Regulatory Element Binding Protein 1c (SREBP-1c) is regulated by glucose at the transcriptional level in MIN6 β cell line.
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Background and Aims: SREBP 1c plays an important role in the regulation by glucose and insulin of lipogenic gene transcription in liver and adipose tissue. Glucose also enhances the expression of similar genes in pancreatic islet- β cells, but the role of SREBP 1c in this cell type is unknown. Our principal aim was to explore the molecular basis of the regulation of SREBP 1c gene expression by glucose in the β cell type.

Materials and Methods: Homogenates were derived from the glucose responsive MIN6 β cell line were subjected to RT-PCR and western blotting to quantify SREBP 1c in this cell type. Single cell nuclear microinjection of reporter gene constructs was used to analyse the effect of SREBP 1c on gene expression.

Results: Elevated glucose concentrations (30 versus 3 mM, 24h) increased SREBP 1c mRNA levels (1.6 ± 0.6 -fold; $n = 3$ experiments). Both unprocessed ($58.8 \pm 8.7\%$; $n = 3$ experiments) SREBP 1c protein levels were also increased by high glucose concentrations. Addition of exogenous insulin (20 nM) did not affect these changes. However, high glucose concentrations increased SREBP 1c promoter activity (2.2 ± 0.8 fold; $n = 20$ (3 mM) and $n = 25$ (30 mM) cells; 3 experiments). Microinjection of a dominant negative form of SREBP 1c impaired transcription from the L-pyruvate kinase, acetyl CoA carboxylase, and preproinsulin promoters at elevated glucose (30 mM). Injection of a constitutively active form of SREBP 1c increased transcription from the same promoters at elevated but not low glucose concentrations.

Conclusions: SREBP 1c is expressed in MIN6 cells and it can be regulated by glucose, which acts at the transcriptional level. SREBP 1c could be involved in the regulation of ACC1, L-type pyruvate kinase and preproinsulin gene expression in the β cell. Its enhanced expression during prolonged hyperglycaemia could contribute to lipid deposition in β cells and defective insulin secretion.

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Proteome mediated identification of proteins involved in β -cell maturation.

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Background: Type 1 insulin-dependent diabetes mellitus (T1DM) is associated with a specific destruction of the pancreatic β -cells in the islets of Langerhans. Increased sensitivity to cytokines, in particular interleukin-1 β (IL-1 β) seems to be an acquired trait during β -cell maturation. We hypothesize that in response to cytokines both protective and deleterious mechanisms are induced, and when the deleterious prevail T1DM develops. To analyze this hypothesis we used two cell-types, which dependent upon cell-culture matures from a glucagons-producing pre- β -cell phenotype (NHI-glu) to an insulin-producing β -cell phenotype (NHI-ins). Previous analysis demonstrated that maturation from the pre- β -cell to the β -cell phenotype is associated with an acquired sensitivity to the toxic effect of IL-1 β . **Aim:** To identify proteins of changed expression level involved in β -cell maturation and acquired sensitivity to IL-1 β by using proteome analysis. **Methods:** 2D-gel-electrophoresis was performed on the two phenotypes followed by computer-assisted comparison of the protein-spots. Protein-spots of altered expression level were identified by mass spectrometry (MS). **Results:** Out of 2,239 detectable, 135 protein-spot showed an altered expression level during β -cell maturation ($N=4$, $p<0.01$). Of these, 74 were down-regulated, 44 up-regulated, 16 were suppressed and 1 was expressed *de novo*. From the 135 protein-spots, 109 different protein identifications were obtained and of these were 26 proteins present in more than one protein-spot reflecting post-translational modification. The identified proteins were assigned in 6 groups according to the known major functions: 1) glycolytic enzymes (8), 2) aminoacid pathway and protein synthesis/degradation (19), 3) energy transduction (11), 4) cytokinesis, nucleic acid synthesis, transcription and nuclear transport (20), 5) chaperones, translocation, protein folding and cellular transport (25) and 6) signal transduction, regulation, growth, differentiation and apoptosis (26). **Conclusion:** Maturation of the pancreatic β -cells is complex involving several interacting mechanisms. The proteins involved and their specific functional importance for β -cell maturation and the acquired sensitivity to cytokines needs to be further elucidated. An understanding of the molecular mechanisms responsible for these processes may yield important information to novel strategies towards intervention, prevention and/or islet transplantation in T1DM.

OP 20

Prediction of Type 1 Diabetes

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Diabetes related autoantibodies in cord blood from children of healthy mothers have disappeared when the child is one-year old.
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Background and Aims: Auto-antibodies found in cord blood in children have been said to predict diabetes later in life. If auto-antibodies are produced by the foetus one would expect to find them in the children still at the age of one year. Our aim is to screen for GAD and IA2 antibodies in cord blood, and then follow-up at the age of one year.

Materials and Methods: ABIS (All Babies in Southeast Sweden) includes 17,000 new-born children who are followed prospectively with blood samples taken at birth and after 1, 2.5 and 5 years. We have determined auto-antibodies in cord blood from 2,518 randomly selected children against Glutamic Acid Decarboxylase 65 (GAD), Thryosinphosphatase (IA-2) by immunoprecipitation of 3H-labelled antigen.

Results: 49 were positive for GAD 65 antibodies and 14 for IA-2 antibodies. Three of them were positive for both. Four of the mothers of children with GAD65 antibodies in cord blood (8.2%) had Type 1 diabetes and 4 mothers to children with IA-2 antibodies (28.6%), but only 0.4% in the antibody negative group. Infection during pregnancy was more common in autoantibody positive (32.7%) than antibody negative (20.1%; $p<0.03$). At one year follow-up of those positive in cord blood all were antibody negative.

Conclusions: As autoantibodies found in cord blood had disappeared when the child is 1 year old they are probably passively transferred from mother to child. Thus antibody screening of cord blood will not be a good method to predict diabetes in the general population.

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DiPiS - Diabetes Prediction in Skåne

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Background and Aims: The aspects of type 1 diabetes etiology suggest that HLA-DQ is the major genetic susceptibility locus which modify risk in response to environmental factors and appearance of islet cell autoimmunity. Dried blood spots have been developed to efficiently screen for genetic factors using PCR amplification. Children who later developed diabetes may have islet cell autoantibodies already in their cord blood. Since these children were born to healthy mothers it was speculated that the children had been exposed to type 1 diabetes pathogenetic processes already in utero. The aims are 1) to screen all newborns in Skåne; 2) to relate their HLA types and other genetic factors to autoantibodies against GAD65 and IA-2, alone or in combination to determine the positive predictive value for type 1 diabetes. **Materials and Methods:** All mothers and their newborns in the Skåne region of Sweden are currently screened at birth. Skåne has 1.4 million inhabitants and about 10,000 children are born per year. More than 100 children are diagnosed with type 1 diabetes per year. Dried blood spots are prepared at birth from mother and child for HLA typing and autoantibody analysis. Time-resolved fluorometry-based HLA-DQB1 typing on dried blood spots are used to assess type 1 diabetes risk alleles. Following elution from the dried blood spots, GAD65 and IA-2 autoantibodies (Ab) are determined in standardized radioligand binding assays. Both antibodies are first analyzed simultaneously. Samples with high binding index are re-analyzed individually. **Results:** A total of 2019 children were born during the first three months of the study. The number of children with high risk HLA DQ alleles amounted to 6.0% for heterozygous DQB1*02/0302 (02 includes the DR7 associated 0201), 8.8% 0302/X (X is excluding 0602, 0603, 0301 and 02) and 15.3% 02/X (X is excluding 0602, 0603, 0301 and 0302). The simultaneous assay of GAD65Ab and IA-2Ab revealed 60 (3%) samples that required analysis of both autoantibodies. High titer GAD65Ab (>50 U/ml) were found in 17 (0.8%) children and IA-2Ab (>15 U/ml) in 4 (0.3%) children. Double positive GAD65Ab and IA-2Ab were found in one (0.05%) mother-child pair, neither subject had high risk HLA-DQ alleles. Levels of autoantibodies were higher in cord than in the mothers blood at delivery. In 5/17 mother-child pairs, GAD65Ab were detected in the cord but not in the mothers blood sample. Among the 17 GAD65Ab positive children 9 had a DQ 02 allele, 4 a 0302 allele and 9 the non-risk alleles 0602, 0603, or 0301 (n.s.). **Conclusions:** Dried blood spots represents an efficient way to screen newborns and their mothers for both HLA genetic factors and autoantibodies to predict the risk of developing type 1 diabetes.

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IMPORTIN BETA: A NOVEL AUTOANTIGEN IN TYPE 1 DIABETES IDENTIFIED BY SCREENING RANDOM PEPTIDE LIBRARIES ON PHAGE WITH DIABETIC SERA

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Background and Aims: In order to identify novel autoantigens in Type 1 diabetes we screened random peptide libraries on phage (RPLs) with Type 1 diabetic sera. With this method, we identified five disease-specific 'minotopes' displayed on phage (phagotopes). Their sequence did not correspond to any known protein on Databases. We initially characterised one phagotope (CH1p) as an epitope of human osteopontin, an autoantigen expressed within the somatostatin cells of human islets. The aim of this study was to characterise a second phagotope, 195Dyn, which was reacting with 20% newly diagnosed diabetic sera and none of the normal controls.

Materials and Methods: In order to characterise phagotope 195Dyn, we raised a specific rabbit antibody against it, which was employed in immunohistochemistry, in the screening of a lambda gt11 cDNA library from human islets and in Western Blot. **Results:** The 195Dyn minotope was detected in human islets according to a classical ICA staining (whole pattern). The screening of the lambda gt11 cDNA library identified a specific clone, whose sequence corresponded to human importin beta. In Western Blot of human osteosarcoma cell extracts, the anti-195Dyn antibodies identified a protein of approximately 90kD corresponding to that produced by anti-importin beta antibodies. In preliminary studies, antibodies against the protein were demonstrated by ELISA in 10/10 human diabetic sera and 1/10 normal controls, and radioimmunoassay with the recombinant protein with a large number of diabetic sera and controls is at present under investigation.

Conclusions: In summary, RPLs proved to be successful in identifying another novel islet-related autoantigen (importin beta), whose significance in disease remains to be established.

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THE IA-2 INTRACELLULAR FRAGMENT (a.a.761-964) IS THE MOST SENSITIVE MARKER OF IA-2 AUTOIMMUNITY IN TYPE 1 DIABETES

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Background and Aims: Recently, we successfully utilized the strategy of displaying peptides on the surface of bacteriophages to screen a diabetes specific cDNA library. Such methodology, applied to fragment IA2/512bdc of the tyrosine-phosphatase 2 (a.a.256-556:630-979) allowed us to identify two major IA-2 autoreactive epitopes in the intracellular domain of the protein (a.a.761-964 and a.a.929-979). The larger clone (a.a.761-964) was found to be autoimmune target of all 512bdc autoantibody-positive type 1 diabetic sera investigated in that study, suggesting that this region contains the main autoantigenic repertoire of tyrosine-phosphatase 2. Aim of this work was to analyze the autoantibody response to IA-2 (a.a.761-964) construct in comparison with that of other five IA-2 fragments (a.a.256-760, 761-928, 929-979, IA2/512bdc, 601-979) and full-length protein (a.a.1-979). **Patients and Methods:** We tested sera from 39 first degree relatives of type 1 diabetic patients (20m,19f), ICA and/or GAD65Ab and/or IAAb positive, who subsequently developed type 1 diabetes (16/39 sequentially followed until diagnosis), 131 randomly selected newly diagnosed type 1 diabetic patients (74m,57f) and 100 normal controls (53m,47f). Autoantibodies to IA-2 fragments were detected by a quantitative radioimmunoassay using [35S]-methionine. Values above 99th percentile of normal controls were considered positive and calculated separately for each IA-2 fragment.

Results: 31/39 type 1 diabetic first degree relatives had autoantibodies directed against at least one IA-2 fragment, in particular 31/31 against IA-2(761-964) construct, 19/31 vs IA-2(256-760), 18/31 vs IA-2(761-928), 23/31 vs IA-2(929-979), 27/31 vs IA-2/512bdc, 30/31 vs IA-2(601-979), 29/31 vs IA-2(1-979). Interestingly, as for the 16 prediabetic patients followed up before diagnosis, in all 16 of them the first autoantibodies to appear were directed against fragments IA-2(761-964) and IA-2(601-979). Among newly diagnosed type 1 diabetic patients 84/131 had autoantibodies directed against IA-2(761-964) construct, 42/131 vs IA-2(256-760), 32/131 vs IA-2(761-928), 59/131 vs IA-2(929-979), 78/131 vs IA-2/512bdc, 78/131 vs IA-2(601-979) and 75/131 vs IA-2(1-979).

Conclusions: These data confirm IA-2(761-964) domain as a dominant target of IA-2 autoimmunity in prodromal-phases and at diagnosis of type 1 diabetes, strongly suggesting the application of this construct as autoimmune marker in disease prediction and prevention studies.

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Enterovirus infections and the risk of Type 1 diabetes

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Background and Aims: We have previously found that enterovirus infections are associated with increased risk for Type 1 diabetes in prospective studies. In the present study we analysed further this association by studying extended number of children who were followed from the birth and who turned positive for diabetes-associated autoantibodies or developed clinical diabetes during the observation.

Materials and Methods: The study subjects participated the Finnish Diabetes Prediction and Prevention Study (DIPP). In this study all newborns are first screened for diabetes associated HLA-DQB1 alleles and those with high risk alleles are invited to follow-up. Enterovirus infections were diagnosed by serology and RT-PCR from serum samples taken during the follow-up of 41 case children who developed type 1 diabetes associated autoantibodies or clinical diabetes and 196 control children matched for the time of birth, gender and HLA-DQB1 alleles.

Results: Enterovirus infections were more frequent in case children than in control children: 24% (59/248) vs. 16% (183/1114) of the follow-up sample intervals indicated infection, respectively (p=0.004). The average enterovirus antibody levels were also higher in case children than in control children (p=0.003 for coxsackievirus B4 IgG). To study further possible causal association we analysed if there was any temporal association between the induction of autoantibodies and enterovirus infections. Enterovirus infections were particularly frequent during the 6 months period preceding the first detection of autoantibodies: 51% (21/41) of the case children compared to 28% (55/196) of the control children had infection at that time (odds ratio [OR] 3.0, 95% CI 1.4-6.4). Enterovirus RNA was found in 17% of the case children and 3% of the control children during this period (OR 7.7, 95% CI 2.1-29.2). There was no difference in the frequency of adenovirus infections between the case and control groups (p=0.8).

Conclusions: This data confirm our previous findings and support the hypothesis that enterovirus infections are a risk factor for type 1 diabetes.

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IA-2 AUTOANTIBODIES BETTER PREDICT IMPENDING TYPE 1 DIABETES THAN MULTIPLE ANTIBODY POSITIVITY

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Background and Aims: Multiple antibody positivity is generally considered as the main predictor for progression to clinical onset of type 1 diabetes. We compared its predictive value for impending type 1 diabetes with that of positivity for IA-2 antibodies. **Materials and Methods:** Siblings (n=1724; median age [range]: 16 [0-39] years) of type 1 diabetic patients were followed for a median (range) period of 24 (0-131) months. Antibodies against islet cytoplasm (ICA) were measured by indirect immunofluorescence and antibodies against glutamate decarboxylase (GADA), IA-2 protein (IA-2A) and insulin (IAA) by radioligand assays. Antibody-positive siblings were HLA DQ genotyped. Survival analysis was used to assess progression to diabetes. **Results:** On initial sampling 4.2% of siblings were positive for ICA, 5.6% for GADA, 1.7% for IA-2A, 5.5% for IAA, 11% for at least 1 antibody type and 2.0% for at least 3 types. Twenty-five siblings developed diabetes after a median (range) follow-up of 13 (2-77) months. Their antibody positivity during the preclinical phase increased from 52 to 76% for ICA, 64 to 68% for GADA, 56 to 72% for IA-2A and 48 to 64% for IAA. They were positive for at least 1 antibody in 92% of cases at first sampling and in 100% at clinical onset and for at least 3 antibodies in respectively 44 and 68% of cases. Progression to clinical onset tended to be associated with the number of antibody positivities preclinically, reaching 44% within 4 years for n=3 (P<0.001 vs n=1); however, this progression was not significantly different for subjects with positivity for 2, 3 or 4 antibodies. Positivity for 2 or 3 antibodies other than IA-2A did confer less than 15% progression within the next 6 years (P<0.002 vs IA-2A positivity). Positivity for IA-2A was associated with 70% progression to diabetes within 4 years vs 1% in absence of IA-2A (P<0.001). In IA-2A positive siblings the progression rate tended to increase with IA-2A levels (P=0.06 for highest tertile vs the rest) and with the presence of the HLA DQ2/DQ8 high risk genotype (P=0.08 vs non [DQ2/DQ8]) but not with age or the number of other antibodies present. In 87% of initially IA-2A positive subjects positivity persisted during follow-up. **Conclusions:** In siblings of type 1 diabetic patients IA-2A positivity better predicts impending clinical onset than multiple antibody positivity. IA-2A levels and HLA DQ status may further refine prediction. These findings may facilitate the enrolment of subjects with homogeneously high risk for diabetes in prevention trials, hereby reducing the sample sizes needed.

OP 21

Advanced Glycation End-Products

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Pyridoxamine inhibits formation of advanced glycosylation and lipoxidation end-products and retards development of nephropathy in hyperlipidemic and diabetic rats.

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Background and Aims: We evaluated the effect of pyridoxamine (PM), an inhibitor of formation of advanced glycosylation and lipoxidation end-products (AGE/ALEs), on the formation of AGE/ALEs and development of nephropathy in streptozotocin (STZ)-diabetic Sprague Dawley (n=12) and obese, pre-diabetic Zucker (fa/fa; n=7) rats.

Materials and Methods: PM was administered in drinking water at 1-2 g/L for 7 months. Plasma glucose (Glc), glycated hemoglobin (GHb) and plasma triglycerides (TG) were measured by commercial kits, and 24-hour urinary albumin (UA) by ELISA. Glycation (fructoselysine (FL)), AGE (pentosidine), ALE (malondialdehyde-lysine (MDA-Lys)) and AGE/ALEs (carboxymethyl- and carboxyethyl-lysine (CML/CEL)) were measured by HPLC and GC/MS.

Results: Glc increased from 5 to 25 mM, and GHb from 7 to 13% in STZ rats; fa/fa rats remained normoglycemic. PM had no effect on glycemia in either STZ or fa/fa rats. FL increased ~5-fold in STZ, compared to non-diabetic rats. TG increased from ~75 mg/dL in non-diabetic and lean controls to 400 and 750 mg/dL in STZ and fa/fa rats, respectively, and was decreased by ~50% toward control values by PM (p<0.01). Pentosidine, MDA-Lys, and CML/CEL increased 2-5 fold in skin collagen of both STZ and fa/fa, compared to control rats, and, except for pentosidine, were decreased ~50% toward control values by PM (p<0.01). UA increased to a mean of 43 and 31 mg/24 hr in STZ and fa/fa rats, and was decreased by PM to 13 and 6 mg/24 hr (p<0.01), vs. control values of 2-3 mg/24 hr.

Conclusions: Dyslipidemia, even in the absence of hyperglycemia, causes a significant increase in AGE/ALE formation in tissue proteins. PM was a potent inhibitor of both hyperlipidemia and AGE/ALE formation, and also inhibited the progression of nephropathy in STZ-diabetic and Zucker pre-diabetic rats. PM should be useful for treatment of nephropathy in both type 1 and type 2 diabetes.

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FOOD ADVANCED GLYCATION ENDPRODUCTS INDUCE ACTIVATION OF PLATELETS BY INCREASING EXPRESSION OF RECEPTORS FOR AGE
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Background and Aims: Advanced Glycation Endproducts (AGE) can induce platelet activation, the key event in atherothrombosis superimposed to coronary artery disease, but it remained unknown whether an AGE-specific, receptor-related signalling mechanism (RAGE) in human platelets exists that can be triggered by food AGE.

Materials and Methods: The effect of AGE derived from Coca-Cola, Pepsi-Cola and Red Star Cola, Cocoa Sarotti and Nescafé purified by lysozyme affinity chromatography was examined at various concentrations (1-300 µg AGE-peptide/ml = 0.7 - 210 AGE U/ml) for 0.1 - 2 h in vitro on freshly isolated blood platelets (10⁷/µl) from fasted diabetic (n=31) and nondiabetic (n=10) subjects according to Düsseldorf III protocol. Platelet activation, determined as expression of CD62, CD 63, CD 41 and receptor bound fibrinogen (RF) at the platelet surface membrane, was measured by FACS-analysis using specific antibodies. The presence of RAGE in platelet membranes from 11 subjects (9 diabetic, 2 non-diabetic) was examined by Western Blot from platelet lysates and by flowcytometric (FACS)-analysis of freshly isolated platelets. Both methods used a specific antibody against the N-terminus of RAGE (Santa Cruz Biotechnology). The effect of Cola-AGE (7 U/ml) on RAGE expression was studied by incubating platelets (10⁷/µl) for 15 min. 37°C. **Results:** RAGE was present in platelet membranes of all subjects, independently by Western Blot and FACS-analysis. Cola-AGE increased RAGE expression at the platelet surface membrane from 14.9±3.3% up to 30.1±6.7% positive platelets (p<0.01). All food-AGE stimulated the expression of CD 41, 62, 63 and RF at the platelet surface membrane from diabetic and nondiabetic subjects as a function of concentration and time, reaching maximal stimulation at 15- min. at 10-30 µg AGE-peptide/ml with a significant increase in platelet aggregation and a concomitant decrease in platelets/µl. **Conclusions:** The increased RAGE expression in human platelets offers a signalling mechanism for food AGE, in concentrations that occur in vivo after an AGE-rich meal, that could favor postprandially the precipitation of acute ischemic events.

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MOLECULAR ANALYSIS OF THE EFFECTS OF RAGE PROMOTER POLYMORPHISMS ON GENE FUNCTION

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Background and Aims: The Receptor for Advanced Glycation End-products (RAGE) has been implicated in the pathogenesis of diabetic vascular complications. AGE/RAGE interaction results in expression of procoagulatory and proinflammatory genes leading to an altered vascular phenotype. Blockage of AGE/RAGE has been demonstrated to reverse these effects and to prevent the development of atherosclerosis in animal models. The hallmark of RAGE in pathogenic situations is the increased cellular expression found. It is possible that polymorphisms within key promoter regions may influence RAGE expression and its effects. We previously identified the -374 T/A and -429 T/C polymorphisms, of which the -429 C allele was found to associate with retinopathy. In this study we investigated the functional effect of these polymorphisms. **Materials and Methods:** We have investigated the effects of the polymorphisms on promoter function by reporter gene studies and transcription factor binding assays. **Results:** In reporter gene studies, the -429 C and -374 A alleles resulted in a 2 to 3 fold increase in expression. We are currently investigating this further by expressing these variants in other cell types, with/without AGE stimulation. Investigation of the effects of these polymorphisms on transcription factor binding was performed with nuclear extract from the monocytic cell line U937 and HepG2 cells. Although no clear differences were seen between the T and C alleles for the -429 polymorphism, the introduction of the rarer A allele of the -374 polymorphism completely abolished the DNA:protein complex using nuclear extract from both U937 and HepG2 cells. Together with the reporter gene data, this suggests the disruption of a repressor domain in these cell types, possibly leading to increased RAGE expression. Analysis of the polymorphic regions for transcription factor binding sites by TRANSFAC implicated a number of repressor-like factors. We are therefore currently characterizing any binding motifs involved using DNaseI footprinting, which will help identify the factor(s) involved.

Conclusions: The -429 and -374 polymorphisms appear to have a functional effect on RAGE transcriptional regulation. Further studies are required to characterise which transcription factors are affected by these polymorphisms and to assess the influence on RAGE gene expression in situ. This data should provide new information on the role of RAGE and its genetics in diabetic vascular disease.

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ELEVATION OF SERUM AGE-PEPTIDE IN PATIENTS WITH DIABETIC NEPHROPATHY DETECTED BY FLOW INJECTION ASSAY

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Background and Aims: Advanced glycosylation end products (AGEs) may play a central role in the pathogenesis of diabetic nephropathy (DN) due to hyperglycemia. Clinical quantitation of AGEs especially low molecular mass AGE-peptide in serum may serve as a useful marker for monitoring pathological process and progression of DN. In the present study, we detected the serum AGE-peptide levels in the healthy control people and diabetic patients with or without DN by flow injection assay (FIA).

Materials and Methods: Serum samples were obtained from 54 age-matched healthy nondiabetic subjects and 126 patients with DM. DM patients were divided into four groups: 35 normoalbuminuria (N)(UAER<30mg/24h); 33 microalbuminuria (Mi)(UAER>30mg/24h and UAER<300mg/24h); 30 macroalbuminuria (Ma)(UAER>300mg/24h); 28 overt proteinuria with insufficient renal function(RF). A flow injection system was set up by using HPLC to detect AGE-peptide.

Results: The coefficient of variance for intra-assay and inter-assay were 1.21% and 6.35%, respectively; The range of recoveries was 94.88% ~ 101.89%; The serum AGE-peptide level was significantly elevated in four groups of diabetic patients (N:1.8±0.6, Mi:1.8±0.4, Ma:2.1±0.9 and RF:3.5±1.3 U/ml, respectively) as compared with control group (1.4±0.5 U/ml, P<0.01-0.0001); Level of AGE-peptide was positively correlated with serum creatinine(r=0.71, P<0.0001), 24 hour urinary protein(r=0.87, P<0.0001) and urinary albumin excretion(r=0.59, P<0.0001).

Conclusions: 1. FIA might be a reliable method for measuring the serum AGE-peptide. 2. The serum AGE-peptide were significantly elevated in the patients with DN and might relate to the severity of DN.

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Fluorescent advanced glycation endproducts, argpyrimidine and pentosidine, in lens proteins of diabetic and healthy control human subjects

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Background and Aims: The accumulation of advanced glycation endproducts (AGEs) have been implicated in diabetic complications. One of the key sites of AGE accumulation is the lens where AGEs may be linked to colouration of cataract. Non-tryptophan fluorescence has been used to determine AGEs in lens proteins. Argpyrimidine, formed from methylglyoxal, has recently been found to be a key fluorescent AGE. Pentosidine is a further well-studied fluorescent AGE formed from pentose sugars. In this study, we compare the concentrations of argpyrimidine and pentosidine in lens proteins of diabetic and normal healthy human subjects.

Materials and Methods: Lens were extracted from 45 human subjects: 25 normal healthy controls with age of 66 ± 17 years (15 male, 10 female), and 18 subjects with diabetes mellitus with an age of 71 ± 9 years (6 male, 12 female); $P > 0.05$. Four normal controls were non-cataractous; 24 were cataractous with 16 of mild and 6 severe colouration. All lens of diabetic subjects were cataractous with 11 of mild and 7 severe colouration. Lens proteins were de-lipidified, washed by ultrafiltration and hydrolysed enzymatically - to avoid acidic degradation of AGEs. Argpyrimidine and pentosidine were assayed by HPLC with fluorescence detection (ex. 320 nm, em. 385 nm). Arginine content of lens hydrolysates was determined by derivatisation with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) and HPLC with fluorescence detection (ex. 245 nm, em. 395 nm).

Results: The concentration of argpyrimidine in lens proteins was (mean \pm SD, mmol/mol arginine): normal controls 0.764 ± 0.353 , diabetic subjects 0.726 ± 0.339 ($P > 0.05$). The concentration of pentosidine in lens proteins (median, range, mmol/mol arginine): normal controls 0.0054, 0.0010 - 0.0160; diabetic subjects 0.0031, 0.001 - 0.0353 ($P > 0.05$). Pentosidine was less than the limit of detection (0.0004 mmol/mol arginine) in 20 samples. Therefore, argpyrimidine content of human lens was > 20 fold higher than pentosidine and was a major AGE fluorophore. There was no significant difference in argpyrimidine or pentosidine content of lens with mild and severe colouration ($P > 0.05$).

Conclusions: Argpyrimidine is a major AGE fluorophore in lens was present at ca. 0.7 mmol/mol arginine. Pentosidine content of lens proteins was > 20 fold less. Lens with mild and severe colouration both contained argpyrimidine and pentosidine.

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Association between serum advanced glycation endproduct concentrations and mild cognitive impairment in elderly diabetic patients

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Background and Aims: Advanced glycation endproducts (AGEs) have been postulated to be involved in the mechanism for Alzheimer disease as well as diabetic complications. AGE might affect cognitive function through the action of neurotoxicity. However, few studies have been conducted to assess the relationship between AGE and cognitive function in diabetic patients.

Materials and Methods: Three hundred thirty six elderly diabetic outpatients (125 men, 216 women) with a mean age of 74 years (duration of diabetes; 14 years) were selected from 463 participants in a longitudinal study on QOL of elderly diabetes mellitus. Serum AGE was measured with enzyme immunoassay (EIA) using an antibody that crossreacted with carboxymethyllysine (CV<5%). Several domains of cognitive function were assessed using the WAIS-R (digit symbol substitution, backward digit span, picture arrangement, similarity), Stroop test, Benton visual retention test, and mini-mental state examination test (MMSE). The associations between serum AGE and other variables were assessed by Spearman's rank correlation.

Results: Serum AGE levels in elderly diabetic patients significantly correlated with age ($r = 0.23$, $P < 0.001$), duration of diabetes ($r = 0.23$, $P < 0.001$), and HbA1c ($r = 0.22$, $P < 0.001$). Serum AGE concentrations were significantly associated with the score of digit symbol substitution (WAIS-R) ($r = -0.15$, $P < 0.05$), Stroop test ($r = 0.18$, $P < 0.05$), MMSE ($r = -0.10$, $P < 0.10$), but not Benton visual retention test, and the other tests of WAIS-R. The results suggest that the AGE may affect attention, concentration, and speed of mental processing. However, there was no significant association between serum AGE and the presence or number of cerebral infarction (defined as localized T2-weighted high, T1-weighted low lesions by brain MR images). The association between serum AGE and digit symbol substitution test score remained significant after adjustment for HbA1c using multiple linear regression analysis.

Conclusions: High serum AGE concentrations in elderly diabetic patients were associated with the impairment of attention and speed of mental processing independent of the presence of cerebral infarction.

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Nephropathy: Clinical

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Insulin Mediated Increase in Angiotensinogen Expression and Angiotensin II Secretion from Human Abdominal Adipose Tissue

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Background and Aims: Angiotensinogen is an important regulator of blood pressure and is known to be increased in obesity and type 2 diabetes. Previous studies have demonstrated the presence of both angiotensinogen and active metabolite angiotensin II in human adipose tissue.

Materials and Methods: In this study we investigated the influence of varying insulin doses on both the protein and mRNA expression of angiotensinogen and the secretion of angiotensin II in human female subcutaneous (Sc) abdominal fat ($n=12$). Isolated Sc adipocytes were treated with varying doses of insulin (1nM-1000nM) for 48hrs. Following treatment, the adipocyte medium, including secreted products and adipocytes were harvested for protein and mRNA levels. Western blotting was performed on the protein extracted from the adipocytes to determine angiotensinogen expression. ELISAs were performed on the collected medium to determine angiotensin II expression. Angiotensinogen mRNA levels were also assessed by quantitative real time PCR.

Results: Increasing doses of insulin (Ins) raised angiotensinogen protein expression in a dose dependent manner (Control 1.0 ± 0.0 , (mean \pm SE), protein expression measured relative to control; Ins 1nM 1.13 ± 0.1 ; Ins 10nM $1.37 \pm 0.14^*$; Ins 100nM $2.1 \pm 0.3^{**}$; Ins 500nM $4.1 \pm 0.83^{**}$; Ins 1000nM $5.2 \pm 0.7^{**}$; $^*p < 0.05$, $^{**}p < 0.01$). Insulin also raised angiotensin II secretion in a similar pattern to angiotensinogen protein expression (Control 214 ± 12.6 pg/ml, Ins 1nM 266.3 ± 12.5 pg/ml; Ins 10nM 358.8 ± 21.1 pg/ml; Ins 100nM 459 ± 22.7 pg/ml ** ; Ins 500nM 759.5 ± 53.3 pg/ml *** ; Ins 1000nM 2122.0 ± 116.7 pg/ml *** ; $^{***}p < 0.001$). However assessment of insulin treated adipocytes by mRNA analysis in female subjects revealed no significant alteration in gene expression compared to control (Control: delta Cycle threshold (dCt) 17.36 ± 0.29 (mean \pm SE); Ins 1nM: dCt 16.91 ± 0.38 ; Ins 100nM: dCt 17.04 ± 0.14 ; $p = N.S.$).

Conclusions: Increasing insulin doses stimulates both angiotensinogen and angiotensin II production, which appears unrelated to mRNA expression, in insulin-treated female subcutaneous adipocytes. In this study protein analysis indicates that insulin may be an important factor for affecting obesity-related hypertension.

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Decrease in Age Adjusted IGF-I and Kidney Volume Decrease at Eight Year Followup of Type 1 Diabetics

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Background and Aims: In type 1 diabetes an initial increase in kidney volume and filtration phase turn later into a progressive decline. The aim of this study was to assess the changes over 8 years in kidney volume and to correlate them to renal function. Furthermore the relation to IGF-I (insulin-like growth factor-I) and one of the IGF binding proteins - IGFBP-1 was investigated.

Materials and Methods: Kidney volume was assessed by single-photon emission computed tomography (SPECT), GFR by inulin and RPF by PAH clearance. At baseline the patients mean age was 36 ± 1 y with a duration of disease of 13 ± 1 y. **Results:** HbA1C decreased from $8.5 \pm 0.3\%$ to 7.6 ± 0.2 ($p = 0.0041$). Kidney volume decreased significantly between the first to second estimation (492 ± 12 ml to 415 ± 9 ml $p < 0.0001$). No change was observed in GFR or RPF from first to second measurement (110 ± 2 ml/min to 112 ± 2 ml/min, 624 ± 16 ml/min to 650 ± 18 ml/min). IGF-I absolute levels as well as age adjusted IGF-I SD-score were significantly decreased at second control compared to first control (152 ± 7 ug/l vs 188 ± 9 ug/l $p < 0.0001$ and -1.2 ± 0.2 vs -0.9 ± 0.2 $p = 0.0012$, respectively). IGFBP-1 mean levels were 53 ± 3 ug/l at first control and 72 ± 6 ug/l at second control. No correlations were found between IGF-I or IGFBP-1 on the one hand and kidney volume or renal function on the other hand. A negative correlation was found between diabetes duration and the change in kidney volume between the two visits ($r = 0.48$, $p = 0.0067$).

Conclusion: Type 1 diabetics with over 10 years of duration show a decrease in kidney volume over 8 years despite an improved metabolic control and an unchanged GFR. IGF-I absolute levels and the age adjusted IGF-I (SD-score) decreased significantly during follow-up.

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Renoprotective Effects Of Losartan In Diabetic Nephropathy: Interaction with Angiotensin I Converting Enzyme Insertion/Deletion genotype?

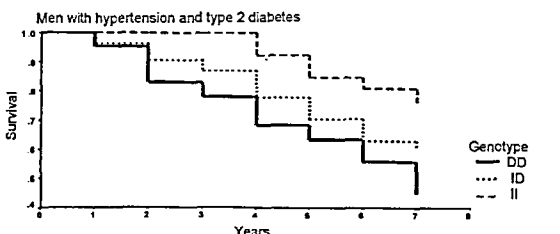
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Background and Aims: Reduced renoprotective effect of Angiotensin I Converting Enzyme (ACE) inhibition has been documented in albuminuric diabetic patients homozygous for the deletion polymorphism of the ACE gene in whom serum ACE is elevated. To overcome this interaction we evaluated the short-term renoprotective effect in diabetic nephropathy of the angiotensin II receptor antagonist Losartan in diabetic patients homozygous for the insertion or the deletion allele. **Material and Methods:** Fifty-four hypertensive type 1 diabetic patients with diabetic nephropathy homozygous for the insertion (I) (n = 26) or the deletion (D) (n = 28) allele of the ACE/ID polymorphism were included. After four weeks of washout, a clinical trial with two treatment periods each lasting two months was performed. In the first period all patients received Losartan 50 mg daily, followed by 100 mg in the second period. Patients and investigators were blinded to ACE genotypes. At baseline and in the end of the treatment periods, albuminuria (ELISA), 24 hours blood pressure (TM2420 A&D) and glomerular filtration rate (GFR) (51Cr-EDTA plasma clearance) were determined. **Results:** At baseline, albuminuria, systolic/diastolic blood pressure and GFR were similar in the two genotype groups, I vs DD: 1123 (821-1537) vs 1210 (886-1655) (geometric mean (95 % CI) mg/24 hours, 156/82 (3/2)(mean (SE) vs 153/80 (3/2) mm Hg and 86 (4) vs 88 (4) ml/min/1.73m², respectively. As expected, concentration of ACE in serum was higher in the DD group compared to the I group, 25 IU/l (I) versus 17 (I) (mean (SE)), respectively (p < 0.05), and did not change during treatment. Both doses of Losartan significantly lowered albuminuria, systolic and diastolic blood pressure and GFR (p < 0.05 versus baseline). Losartan 100 mg was more effective than 50 mg in reducing albuminuria, 51 % (40-61) (95 % CI) versus 33 % (23-42), respectively (p < 0.01). No differences in changes of variables between the I and DD groups were observed: Losartan 100 mg lowered albuminuria by 55 % (35-68) and 46 % (28-61), whereas systolic/diastolic blood pressure decreased by 12/6 and 10/4 mmHg in the I and DD group, respectively, NS. **Conclusion:** In contrast to previous studies with ACE inhibitors, our data suggest that Losartan has similar short-term renoprotective effects in albuminuric hypertensive type 1 diabetic patients with ACE I and DD genotypes. However, the long-term renoprotective effects remain to be evaluated.

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THE ACE GENE I/D POLYMORPHISM IS ASSOCIATED WITH HIGHER MORTALITY IN MEN WITH HYPERTENSION AND TYPE 2 DIABETES
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Background and aims: To study the impact of the ACE I/D polymorphism on the total mortality in patients with hypertension and type 2 diabetes mellitus. **Patients and Methods:** The insertion/deletion (I/D) polymorphism of the ACE gene was genotyped in 121 men and 124 women with type 2 diabetes and hypertension that participated in a community based study in primary care. Information on patient mortality after 7.5 years was obtained from the National Mortality Register in Sweden. The association between ACE I/D polymorphism and total mortality was analysed in both sexes with Cox's proportional hazards regression with age as a covariate. **Results:** Fifty men (41.3%) and 40 women (32.3%) had died. In men (RR 3.8 95% CI 1.6-9.1, p<0.003), but not in women (RR 1.2 95% CI 0.5-2.8), carriers of the DD genotype had a higher total mortality compared to carriers of the II genotype.



Conclusions The DD genotype of the ACE gene is associated with a higher total mortality in men with hypertension and type 2 diabetes.

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Renoprotective effect of dual blockade of the renin-angiotensin system(RAS) in type 2 diabetic patients with diabetic nephropathy.

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Background and Aims: Many patients with diabetic nephropathy (DN) have albuminuria>1g/day and blood pressure>135/85 mm Hg ("the resistant patient") despite antihypertensive combination therapy including the maximally recommended dose of ACE inhibitor (ACEi). We tested the concept that such patients might benefit from dual blockade of the renin-angiotensin system (RAS).

Material and Methods: We performed a randomised double blind crossover study with 2 months treatment with Candesartan cilexetil 8 mg o.d. and placebo added on top of previous antihypertensive treatment. We included 18 type 2 diabetic patients with DN resistant to conventional treatment as defined above. All received ACEi treatment, in addition 15 received diuretics, 10 a calcium channel antagonist and 3 a β -blocker. At the end of each treatment period we measured glomerular filtration rate (GFR), 24-hour blood pressure, albuminuria and IgGuria.

Results: Addition of Candesartan to usual antihypertensive therapy induced a mean (95% CI) reduction in albuminuria of 25 (2 to 58) %, p=0.036 (geometric mean (95% CI) from 1764(1225 to 2540) to 1334(890 to 1998) mg/24 h), a mean reduction in fractional clearance of albumin of 35 (9 to 53) %, p=0.016 and IgG of 32 (1 to 54) %, p=0.046, a reduction in 24-hour systolic blood pressure of 10 (2 to 18) mm Hg, p=0.019 (mean (SE) from 148(3) to 138(5) mm Hg) and a mean reduction in GFR of 5 (0.1 to 9) ml/min/1.73m², p=0.045.

Conclusions: Dual blockade of the RAS is renoprotective and reduces blood pressure in type 2 diabetic patients with DN resistant to conventional antihypertensive treatment including maximally recommended doses of ACEi.

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Long-term graft and patient survival after renal transplantation in diabetic patients.

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Backgrounds and Aims: Diabetic patients in dialysis treatment have a high excess mortality compared with non-diabetic patients. In contrast, reports on outcome after renal transplantation have been conflicting. This may be due to the selection criteria's for patients receiving a kidney transplantation excluding patients with severe cardiovascular disease, thereby possibly matching diabetic and non-diabetic patients with respect to cardiovascular risk at the time of transplantation. Our aim was to study graft and patient survival in patients transplanted with a cadaver donor kidney.

Materials and Methods: All patients transplanted at Rigshospitalet in the time period 1990 to 1999. Patients were divided into two groups: Non-diabetic patients (n= 354) and diabetic patients (n=47).

Results: The two groups were similar with respect to age (years): 43±9 vs. 43±14 and sex (M/F (%): 66/34 vs. 66/34). First, 2nd, 3rd or more than 3rd transplantation (%): 77 vs. 92, 18 vs. 6, and 5 vs. 2. The outcome was as follows (non-diabetic vs. diabetic patients): Patient survival (%) at 1 year: 89 vs. 89, at 2 years: 85 vs. 87, at 5 years: 71 vs. 68, at 6 years: 67 vs. 47, at 7 years: 63 vs. 37, at 8 years: 58 vs. 12. Graft survival when censoring for death with functioning graft (%): at 1 year 81 vs. 80, at 2 years 79 vs. 80, at 5 years 70 vs. 75, at 6 years 66 vs. 75, at 7 years 65 vs. 75.

Conclusions: The patient survival was similar in the two groups only for the first 4-5 years after renal transplantation; hereafter survival was poor in the diabetic group. The graft survival (censored) was similar in non-diabetic and diabetic patients in the entire observation period. This is in favour of the hypothesis that the two groups had been similar with respect to the cardiovascular risk at the time of transplantation because of the selection criteria's used. The subsequent poor survival among diabetic patients could be due to the well-known rapid and almost malignant progression of cardiovascular disease in diabetic patients with nephropathy.

OP 23

Clinical Studies of Oral Hypoglycaemic Agents

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ADHERENCE TO ORAL HYPOGLYCAEMIC AGENTS IN TYPE 2 DIABETES: IS THIS A PREDICTOR OF INSULIN REQUIREMENT?
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Background and Aims: Non-adherence is an important cause of poor metabolic control in diabetes. We investigated adherence to oral hypoglycaemic agents (OHA) in type 2 diabetes, and whether patients require insulin as a result of poor adherence to OHAs. **Materials and Methods:** The 'DARTS' diabetes information system and the 'MEMO' database of 17 million drugs dispensed since 1993, for the population of Tayside, Scotland (400,000), were used. Patients aged 35+ years with type 2 diabetes who had 180+ days of exclusive therapy with OHAs (sulphonylureas or metformin), in 1993-1996, prior to insulin treatment were identified. The intended duration of every OHA prescription was calculated from details on the prescription (total amount dispensed and drug regimen). Adherence was derived by dividing total intended duration of OHA therapy by time in study for each patient. It was compared between those who did and did not commence insulin therapy. **Results:** There were 2,537 patients on sulphonylureas (51% male, mean age 67 yrs). 262 commenced insulin. Mean adherence was 93.7%. 63% of patients showed adherence $\geq 90\%$. There was improved adherence in patients who did (mean 100.4%) compared with those who did not (mean 92.9%) commence insulin ($p < 0.001$). There were 1,519 patients on metformin (49% male, mean age 64 yrs). 169 commenced insulin. Mean adherence was 85.4%. 50% of patients had adherence $\geq 90\%$. Mean adherence was 82.0% and 85.8% in patients who did and did not commence insulin ($p = 0.124$). In a logistic regression model, other predictors of insulin therapy were age, diabetes duration, co-prescribing of OHAs and time in study. **Conclusions:** Adherence to OHA in patients with type 2 diabetes is sub-optimal, but there is no evidence that patients require insulin as a result of poor adherence to OHAs.

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Effects of rosiglitazone on insulin resistance in HIV infected patients under treatment with protease inhibitors
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Background and Aims: HIV-1 protease inhibitor treatments are associated with a syndrome of lipodystrophy, hyperlipidaemia and insulin resistance. The aim of the study was to determine whether reducing hyperinsulinaemia with rosiglitazone would improve the clinical profile in HIV patients under treatment with protease inhibitors.

Materials and Methods: Patients were eligible for the study if they had impaired glucose tolerance (IGT) with insulin resistance, characterized by fasting insulin concentration greater than 20 mIU/ml. The patients were randomly assigned to receive 4mg qd rosiglitazone (rosiglitazone group, n=20) or no treatment (control group, n=20). Both groups matched for age, sex, BMI, duration of HIV infection, treatment and biochemical values.

Results: After 2 months of treatment, the fasting insulin in our study group declined from 37.4 ± 8.4 to 17.4 ± 4.0 mIU/ml ($p < 0.01$), the fasting glucose from 6.7 ± 0.23 to 4.3 ± 0.08 mmol/l ($p < 0.05$). This improvement in insulin secretion could be clearly shown when the sums of insulin concentrations after OGTT were compared: 322 ± 22 mIU/ml after treatment with rosiglitazone to 544 ± 54 mIU/ml before treatment ($p < 0.01$). The mean serum triglyceride concentration dropped by 23% in rosiglitazone group. There were no changes in the study parameters in our control group.

Conclusions: Rosiglitazone (4mg qd) was well tolerated in HIV patients under treatment with protease inhibitors. It improves the glycaemic control and insulin resistance and significantly reduced serum triglyceride concentration. It remains to be demonstrated whether restoring insulin levels to normal in these patients will reduce the risk of coronary heart disease.

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Repaglinide is well tolerated and effective in Type 2 diabetes complicated by renal impairment
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Background and Aims: Repaglinide is a novel insulin secretagogue characterised by a rapid onset and short duration of action, hepatic metabolism and biliary excretion. It therefore poses a theoretically low risk of hypoglycaemia in patients with renal disease, in contrast to long-acting, renally-excreted sulphonylureas. **Materials and Methods:** The safety and efficacy of prandial repaglinide was assessed in 281 patients with Type 2 diabetes with or without renal impairment in a multinational, open-label trial. Patients were monitored on their existing antidiabetic medication during a 6-week run-in period, after which they switched to a repaglinide treatment phase (1-4 weeks' titration, 3-months' maintenance therapy), with 84% completing. Patients were stratified by creatinine clearance (CLCR) into the categories of: normal renal function (CLCR > 80 ml/min, n = 151), mild renal impairment (CLCR: 60-80 ml/min, n = 64), moderate renal impairment (CLCR: 40-60 ml/min, n = 44), severe renal impairment (CLCR: 30-40 ml/min, n = 12) and extreme renal impairment (CLCR: 20-30 ml/min, n = 10). **Results:** In the run-in period, the percentage of patients reporting hypoglycaemia correlated significantly with the severity of renal impairment ($p = 0.007$), but this pattern was not seen during repaglinide treatment ($p = 0.074$). There was no apparent difference in the type and severity of adverse events reported during the run-in and repaglinide treatment periods. The number of patients reporting adverse events did not relate significantly to renal status. There were no significant between-group differences in terms of the effect that repaglinide had on HbA1c, FBG, total cholesterol, HDL-cholesterol and triglycerides. Glycaemic control during repaglinide treatment was at least as good as that seen during previous antidiabetic medication: HbA1c remained unchanged in the normal, mild and moderate renal impairment groups, increased by $0.3 \pm 1.0\%$ points in the severe renal impairment group and decreased by $0.6 \pm 0.5\%$ points in the extreme renal impairment group. Only minor changes were detected in lipid profiles. Patients with severe and extreme renal impairment tended to require lower final doses of repaglinide to reach glycaemic targets compared to patients with less severely impaired or normal renal function ($p = 0.032$). **Conclusions:** Repaglinide is well tolerated and effective in patients with Type 2 diabetes complicated by renal impairment. When titrated carefully, it is a suitable treatment choice, even for those with severe degrees of impairment.

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EVIDENCE OF A POTENT ANTI-INFLAMMATORY EFFECT OF ROSIGLITAZONE IN MONONUCLEAR CELLS
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Background and Aims: We have recently demonstrated a potent anti-inflammatory effect of troglitazone, a peroxisome proliferator-activated receptor γ (PPAR γ) agonist with some PPAR α activity. We have now investigated a similar effect of rosiglitazone, a specific PPAR γ agonist.

Materials and Methods: Eleven non-diabetic obese patients were given rosiglitazone 4 mg daily for 6 weeks. Fasting blood samples were obtained at 0, 1, 2, 4, 6 and 12 weeks (6 weeks after cessation of the drug). Generation of reactive oxygen species (ROS) and expression of the p47^{phox} subunit of NADPH oxidase were measured in mononuclear cells by chemiluminescence and western blotting, respectively. Levels of monocyte chemoattractant protein 1 (MCP-1) and C-reactive peptide (CRP) were measured by ELISA. Nuclear factor κ B (NF κ B) was measured in nuclear extracts by electrophoretic mobility shift assay (EMSA). Post-ischaemic flow mediated brachial arterial dilatation was measured by a Hewlett-Packard Ultrasonograph by standard techniques.

Results: Blood glucose levels did not change. Brachial arterial reactivity as reflected in post-ischaemic flow mediated dilatation increased from 4% to 10% ($p < 0.01$) at 6 weeks. NF κ B diminished significantly at 1 week and remained low for 6 weeks. There was a significant fall in ROS generation (by 40% from basal level at 6 weeks; $p < 0.05$), and a reduction in p47^{phox} subunit expression (by 25% from basal level at 6 weeks; $p < 0.003$). There was also a fall in serum CRP by 30% ($p < 0.002$) and MCP-1 by 15% ($p < 0.04$) at 6 weeks. All indices returned toward the baseline at 12 weeks.

Conclusions: We conclude that rosiglitazone exerts a profound suppression of NF κ B-mediated ROS generation. This anti-inflammatory effect is reflected at the cellular and molecular level and in plasma. It also improves vascular reactivity. These observations may have implications for atherogenesis in the long-term.

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EFFECT OF ROSIGLITAZONE ON FFA AND GLUCOSE METABOLISM IN TYPE 2 DIABETIC PATIENTS

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Background and Aims: The mechanism of action of thiazolidinediones (TZD) remains unknown. The present study provides evidence that improved glucose homeostasis following rosiglitazone is related to the TZD's beneficial effect on FFA metabolism. **Materials and Methods:** 29 diet-treated T2DM patients (age=56±2 y; BMI=30.0±0.7 kg/m², M/F=16/13) randomly (double blind) were assigned to receive rosiglitazone (8 mg/day) or placebo for 12 wks. Before and after 12 wks, subjects received a 75g OGTT and 2-step euglycemic insulin (infusion rates=40 and 160 mU/m²-min) clamp with ³H-glucose, ¹⁴C-palmitate and indirect calorimetry. Fat mass and FFM were determined with ³H₂O. **Results:** After 12-wks, rosiglitazone decreased FPG (195±11 to 150±7 mg/dl, p<0.01), mean PG during OGTT (293±12 to 236±9 mg/dl, p<0.01), and HbA_{1c} (8.7±0.4 to 7.4±0.3 %, p<0.01) without change in fasting or OGTT-stimulated plasma insulin conc. Basal endogenous glucose production (EGP) decreased (3.3±0.1 to 2.9±0.1 mg/kg FFM-min, p<0.05) and whole body glucose metabolic clearance rate during the 1st and 2nd insulin clamp steps increased after rosiglitazone (1stMCR: 2.8±0.2 to 3.5±0.2 ml/kg FFM-min, p<0.01; 2ndMCR: 6.7±0.6 to 9.2±0.8 ml/kg FFM-min, p<0.05), despite increases in body weight (86±4 to 90±4 kg, p<0.01) and fat mass (33±3 to 37±3 kg, p<0.01). Fasting plasma FFA (735±52 to 579±49 µEq/l, p<0.01), mean plasma FFA during OGTT (561±33 to 424±35 µEq/l, p<0.01), and basal FFA turnover rate (18.3±1.5 to 15.5±1.2 µEq/kg FFM-min, p<0.05) decreased. No changes in fasting or post-OGTT PG, insulin, or FFA concentrations, basal EGP, glucose MCR or FFA turnover rate occurred in the placebo group. The decrease in FPG after rosiglitazone correlated with the change in basal EGP (r=0.54), 1stMCR (r=-0.66), 2ndMCR (r=-0.49), fasting FFA (r=0.53), and mean FFA during OGTT (r=0.66) (all p<0.01). The decrease in mean PG conc during OGTT correlated with the decrease in basal EGP (r=0.58), 1stEGP (r=0.41), 1stMCR (r=-0.68), 2ndMCR (r=-0.54), fasting FFA (r=0.49), and mean FFA during OGTT (r=0.66) (all p<0.01). **Conclusion:** Rosiglitazone increases hepatic and peripheral tissue (muscle) sensitivity to insulin and decreases FFA turnover despite an increase in body fat mass. The close correlation between the improvement in FFA metabolism and parameters of glucose homeostasis suggests that rosiglitazone's beneficial effects on glycemic control are, in part, mediated by the TZD's effect on fat metabolism.

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EFFECTS OF PIOGLITAZONE ON HDL-CHOLESTEROL LEVELS ARE INDEPENDENT OF CHANGES IN TRIGLYCERIDE

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Background and Aims: Pioglitazone HCl (PIO), a thiazolidinedione antidiabetic agent, improves the high triglyceride (TG) and low HDL-cholesterol (HDL-C) levels characteristic of insulin resistance and type 2 diabetes. To examine whether PIO-mediated increases in HDL-C occur independently of PIO-mediated decreases in TG, we evaluated the correlation (r_s) between the observed changes in HDL-C and TG levels in placebo- and PIO-treated patients with type 2 diabetes. **Materials and Methods:** Patients (n=260) entered a 6-week washout period (no antidiabetic drugs) and were then randomized to either placebo or a forced titration of 7.5/15/30 mg PIO or 15/30/45 mg PIO once daily for 16 weeks in a multicenter, double-blind, placebo-controlled study. **Results:** As expected, PIO therapy produced beneficial changes in both TG and HDL-C levels. PIO-treatment (15/30/45 mg PIO) resulted in an average -12.4±4.79% decrease in TG levels compared with a 1.3±4.78% increase (p=0.0454) in the placebo group; HDL-C levels were increased an average 13.1±2.30% with 15/30/45 mg PIO compared with a 4.3±2.32% increase (p<0.05) in the placebo group. While a statistically significant negative correlation between changes in HDL-C and TG levels was observed in the placebo group (r_s=0.23, p<0.05), increases in HDL-C levels did not significantly correlate with decreases in TG levels with PIO treatment: 7.5/15/30 mg PIO (r_s=0.20) and 15/30/45 mg PIO (r_s=0.15). With PIO treatment groups combined, the median decrease in TG levels was -34 mg/dL. The HDL-C response to PIO was compared in patients whose TG response was greater vs less than this value. The percent change in HDL-C was similar in the two groups, 12.71±2.33% vs 12.27±2.19% (p=0.8937), respectively. **Conclusions:** Overall, these results suggest that PIO may improve HDL-C levels independent its effects on TG.

OP 24

Genetics of Type 2 Diabetes: Genome Scans and Positional Candidates

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Linkage analysis in the Diabetes (UK) Warren 2 sibpair repository supports localisation of a type 2 diabetes susceptibility gene to chromosome 1q21-24. M.I. McCarthy, A.T. Hattersley, M. Walker, G.A. Hitman, J.C. Levy, S O'Rahilly, G.M. Lathrop, N. Simecek, M. Wishart, R. Dhillon, C. Fletcher, T.M. Frayling, A. Bennett, C. Groves, D. Smedley, S. Menzel, S. Wiltshire. Imperial College, London; Exeter University; Newcastle University; Queen Mary College, London; Cambridge University, Radcliffe Infirmary and Wellcome Trust Centre for Human Genetics, Oxford, UK.

Background and Aims: The major objectives of the consortium have been to ascertain and analyse large clinical resources and apply these to aid identification of the main susceptibility variants underlying type 2 diabetes in the UK. One focus has been the completion of a 10cM genome scan using a large affected sibpair resource.

Material and Methods: Genome-wide data are now available for 627 paternity-confirmed sibships (equivalent to 815 affected sibpairs), all of British/Irish origin. Mean (SD) ages of diagnosis in 716 male and 622 female affected sibs were 55.2(8.6) and 55.9(8.8)y respectively: mean BMIs were 27.8(4.3) and 29.9(5.7) kg/m². These pedigrees have been typed for 418 autosomal microsatellite markers, and, following extensive error-checking, genotypes were submitted to non-parametric linkage analysis using GENEHUNTER-PLUS and ALLEGRO. **Results:** Based on these data (before any stratification), promising regions meriting detailed further investigation (LOD>1.2) were identified on chrs 1q, 5q, 7p, 8p (LOD=2.55), 8q and 10q (LOD=1.98), several of which coincide with regions identified in other datasets. Of particular note is evidence for excess allele-sharing on 1q21-24 (LOD=1.50, p=0.004) which coincides with, and replicates, linkages previously highlighted in a number of other ethnic groups (Pima, Amish, Utah Mormon, French). Evidence for linkage in this region has been enhanced in our data by (a) a rise in LOD to 1.98 (p=0.001) after typing additional microsatellites (to ~3cM density); (b) an increase in LOD to 2.38 in early-onset pedigree subsets; (c) evidence for interaction with loci on 5p and 10q (LODs > 3.0; p=0.02 for interaction). Draft genome sequence for the ~20cM region has been assembled and mined using GANESH (in-house genome annotation package) to identify ~133 known genes including several strong candidates eg RXRG, LMNA, KCNJ9) and >9500 SNPs that are substrates for ongoing linkage disequilibrium studies. **Conclusions:** We conclude that these data from the UK genome scan add to the growing weight of evidence that a type 2 diabetes susceptibility locus of worldwide significance maps to the 1q21-24 region and that available genome resources should expedite discovery of the aetiological variant.

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GENOME-WIDE SEARCH FOR TYPE2 DIABETES SUSCEPTIBILITY GENES IN JAPANESE AFFECTED SIBPAIRS

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Background and Aims: The genetic background which predisposes to type2 diabetes in the Japanese population is largely unknown. To search for major susceptibility loci, we conducted a 10-cM genome scan for type 2 diabetes traits.

Materials and Methods: A total of 359 individuals of Japanese origin were ascertained in Japan from 158 families, including 229 affected sibpairs. In 202 males, mean age at diagnosis was 45.9±9.7 yrs and maximal BMI was 26.4±3.0 kg.m⁻². In 157 females, mean age at diagnosis was 48.3±11.1 yrs and maximal BMI was 27.4±3.8 kg.m⁻². Fluorescent microsatellite marker set (ABI Linkage Mapping Set version2, MD10) was used for the primary 10cM scan. Additional 33 markers were typed for 13 candidate genes, mainly transcription factors. Non-parametric multipoint linkage analyses were performed by MLBGH1.0 and MAPMAKER-SIB2.0.

Results : Multipoint analyses showed 6 potentially interesting regions, in 1p36-p32 (MLS = 1.65, MLB = 1.44), 3q26-q27 (MLS = 1.25, MLB = 1.20), 7p22-p21 (MLS = 1.80, MLB = 1.50), 11p14-p13 (MLS = 1.80, MLB = 1.75), 15q13-q15 (MLS = 2.10, MLB = 1.40) and 20q12-q13 (MLS = 1.81, MLB = 1.57). In the subset analysis in young-onset 36 families, in which both sibs of pair developed type2 diabetes before 45 yrs, we observed a suggestive linkage in 15q13-q15 (MLS-unweighted = 3.62, p = 0.00005, MLS-weighted = 2.49, p = 0.00068, MLB = 2.62, p = 0.00026).

Conclusions: These genome scan results suggest that there may be several candidate loci conferring susceptibility for type 2 diabetes in the Japanese population.

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META-ANALYSIS OF EUROPEAN GENOME SCANS TO IDENTIFY PRIMARY REGIONS LINKED TO TYPE 2 DIABETES.

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Background and Aims: Genome screens have proved useful to indicate chromosomal regions that may contain susceptibility genes for complex diseases, such as Type 2 Diabetes. However, significant results from one study are not often confirmed by other studies. Our goal was to perform a meta-analysis of four genome scans conducted in European populations in order to assess evidence for linkage across studies and to identify primary regions of interest to be further explored towards gene identification.

Materials and Methods: Linkage analysis results from four genome screens carried out in 573 British and 143 French nuclear families and in two pedigree samples of Swedish-Finnish origin (58 Botnia I and 338 Botnia II pedigrees) were put together in our GIFT Consortium data base. A meta-analysis of these scans was performed using the genome search meta-analysis (GSMA) method. This method is a non-parametric ranking method which ranks lod scores (or other) statistics within each scan and then compares ranks for a genetic region across the searches. It can thus identify the regions that show consistently increased statistics for linkage.

Results and Conclusions: The strongest evidence for linkage given by the GSMA occurs on chromosome 17 ($p = 0.0015$). Other significant regions which may harbour Type 2 Diabetes genes were found on chromosome 2 ($p = 0.03$), 6 ($p = 0.04$), 12 ($p = 0.04$) and 16 ($p = 0.04$). Borderline significant results were also observed on chromosomes 7 and 10. Linkage analyses of pooled raw data will be further carried out in these regions to assess the values and locations of the peak statistics before undertaking fine mapping.

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LOSS OF ABILITY TO UPREGULATE CALPAIN-10 EXPRESSION IN MUSCLE AFTER 24-HOUR INTRALIPID INFUSION IN SUBJECTS WITH IGT
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Background and Aims: The gene for Calpain 10 has been identified as a candidate gene for type 2 diabetes and the levels of Calpain 10 mRNA in muscle have been found to be lower in type 2 diabetes compared to controls. The aim of this study was to investigate the possible role of Calpain 10 and another member of this group of proteases, Calpain 3, in the prediabetic state of impaired glucose tolerance. **Materials and Methods:** Calpain 3 and -10 mRNA levels were semi-quantified in total RNA from muscle biopsies using Real-time RT-PCR with cyclophilin as an internal standard. Biopsies were obtained from subjects with either normal (NGT, $n=8$) or impaired glucose tolerance (IGT, $n=6$) before and after a euglycemic hyperinsulinemic clamp with prior infusion of Intralipid for 0, 2 and 24 hours. The subjects were matched for sex, age (53 ± 2 vs 57 ± 2) and BMI (32.7 ± 1.4 vs 31.8 ± 1.4) (NGT vs IGT; mean \pm SEM; Mann-Whitney for comparisons between and Wilcoxon within the groups). **Results:** There were no significant differences between the groups in basal or post-clamp Calpain 3 (2.2 ± 0.8 vs 1.0 ± 0.4 ; $p=0.3$ and 2.6 ± 1.6 vs 1.1 ± 0.4 ; $p=0.9$) or Calpain 10 (4.3 ± 1.8 vs 1.6 ± 0.5 ; $p=0.5$ and 3.8 ± 1.3 vs 1.8 ± 0.5 ; $p=0.2$) mRNA levels. Insulin had no significant effect on Calpain 3 or Calpain 10 mRNA levels in either group before Intralipid infusion. However, after the 24-hour lipid infusion the muscle Calpain 10 mRNA content increased significantly in the NGT (from 2.4 ± 1.0 to 7.5 ± 2.4 ; $p=0.043$) but not in the IGT group (from 4.7 ± 2.6 to 3.6 ± 1.6 ; $p=0.9$). Finally, in a multiple regression analysis adjusting for BMI there was a negative correlation between the fasting insulin levels of both groups and the levels of Calpain 10 mRNA post-clamp after the 24-hour lipid infusion ($p=0.0017$, $R^2=0.71$). **Conclusions:** Prolonged exposure of muscle to elevated FFA levels seems to upregulate the expression of Calpain 10 in response to insulin in subjects with NGT. This ability is lost in patients with IGT. The question arises whether increased insulin-stimulated Calpain expression in muscle represents a protection against insulin resistance and IGT.

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Genetic Variation of CAPN10 affects Susceptibility to Type 2 Diabetes in German and Czech Population

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Background and Aims: A genome-wide screen for type 2 diabetes genes in Mexican Americans localized a susceptibility gene, NIDDM1, on chromosome 2. Recently CAPN10, a gene in the NIDDM1 region encoding a non-lysosomal cysteine protease, was associated with both type 2 diabetes and the evidence for linkage. Haplotype combinations including intronic SNPs in CAPN10 are associated with altered mRNA expression, increased risk for type 2 diabetes in multiple racial/ethnic groups, and measures of insulin resistance in non-diabetic subjects. **Materials and Methods:** We investigated the effect of 4 variants (UCSNP-44, -43, -19, and -63) of the calpain 10 gene in 615 type 2 diabetic patients and 244 random controls from German and Czech population. Genotyping was done with common PCR-, Sequencing and ARMS methods. Type 2 diabetes risk (OR) and population attributable risk (PAR) were calculated. **Results:** The number of subjects, mean age and BMI were for German pt./contr. $291/88$, $61.8 \pm 11.3/50.8 \pm 11.9$ years and $24.9 \pm 4.4/28.7 \pm 4.8$ kg/m² and for Czech pt./contr. $324/156$, $58.5 \pm 7.4/18.1 \pm 2.3$ years and $30.1 \pm 5.3/24.3 \pm 4.0$ kg/m². The 112 haplotype was in German pt./contr. $0.06/0.02$ and Czech pt./contr. $0.08/0.06$ population less frequent than in Mexican American pt./contr. $0.23/0.23$ population. The haplotype combination 112/121 is associated with increased type-2-diabetes risk (OR germ./cz./comb. $4.98/2.80/3.36$ -95% CI $0.65-38.0/0.80-9.72/1.18-9.60$) with PAR (germ./cz./comb.) $0.04/0.03/0.05$. In the combined German/Czech group carrier of the rare allele at UCSNP-44 (T504A) ($f=0.11$) in combination with the risk allele at UCSNP-43 showed a trend to a generally increased diabetes risk (2.98 -95% CI $0.35-22.2$). **Conclusions:** The haplotype 112/121 is less common in European than in Mexican American population but carries a approximate 3-4fold increased type-2-diabetes risk. The rare allele at UCSNP-44 (T504A) is a common Caucasian variant and associated with increased type-2-diabetes risk. Together this two variants may count for an 5%-10% proportion of diabetes risk in Caucasian diabetic population. We conclude that variation in the calpain 10 gene appears to affect type-2-diabetes susceptibility in Caucasian population.

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ASSOCIATION OF APM1 (ADIPONECTIN) GENE SNPs WITH TYPE 2 DIABETES AND CHD IN FRENCH POPULATIONS.

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Background and Aims: Adipose tissue is known to secrete proteins implicated in the development of diabetes, obesity and their vascular complications. Our genomewide searches for susceptibility genes to type 2 diabetes (T2DM) and to coronary heart disease (CHD) revealed linkages at chromosome 3q27. In this region is located the APM1 gene encoding ACRP30-adiponectin, an adipocyte secreted protein. Low levels of plasma adiponectin were observed in CHD patients with obesity or T2DM. Administration of adiponectin protects high fat diet mice from obesity and rescues lipotrophic mice from severe insulin resistance. Adiponectin may also modulate inflammation by inhibiting vascular smooth muscle cells proliferation and adhesion molecules expression. APM1 is therefore a good candidate for the metabolic abnormalities associated with T2DM and obesity and for CHD in the context of insulin resistance syndrome.

Material and Methods: we screened 16kb of the gene by direct sequencing in 40 T2DM patients. To perform association studies every detected SNPs were genotyped in 384 obese patients, 310 probands from T2DM families, 189 T2DM patients with or without CHD, 223 non diabetic subjects of diabetic families, and in 377 non obese non diabetic control subjects.

Results: Screening the gene revealed 11 SNPs. A significant association was observed between the SNP A-C in intron 1 and the diabetes status in obese patients ($p=0.009$, OR=3). In the same obese population, two additional SNPs G-C and G-A located in promoter region also displayed a trend toward association with diabetic status. In the group of T2DM patients phenotyped for CHD, we detected under a dominant model an association between CHD and two SNPs: T-G in exon 2 ($p=0.029$, OR=2.2) and A-G in intron 2 ($p=0.019$, OR=2.3).

Conclusion: These data suggest a role of ACRP30/adiponectin in the genetic risk for T2DM especially in obese subjects. Moreover, polymorphisms in APM1/ACRP30 gene may modulate the risk for CHD in T2DM. ACRP30/adiponectin may be a fat-induced metabolic link between obesity and T2DM, which may be in part genetically inherited.

OP 25 Fundamental Aspects of Diabetic Retinopathy

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Endothelial Nitric Oxide Synthase and Vitamin D Receptor Polymorphisms Predict Risk for Severe Diabetic Retinopathy.

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Background and Aims: the loss of pericytes precedes the development of diabetic retinopathy (DR), in part by oxidative stress, probably by over production of nitric oxide (NO). The eNOS4 polymorphism of the endothelial nitric oxide synthase (eNOS) has been associated with diabetic nephropathy, but not DR. The vitamin D (VD) has antioxidant and antiangiogenic properties. Vitamin D receptor (VDR) polymorphisms were associated with type 1 diabetes but have never been studied in patients with DR. To examine whether VDR (Taq I) and eNOS4 polymorphisms are involved in the development of severe DR a case-control study was performed. **Materials and Methods:** 200 unrelated French type 1 diabetic patients of long duration, were randomly selected from a group of 1000 patients (M/F:103/97, age:44.4±12.4, diabetes duration:27.7±10yrs, BMI:24.3±3.4, HbA1c: 8.6±1.3%). eNOS4 was analysed by PCR, and Taq I restriction by PCR followed by digestion with Taq I. DR was assessed by retinal angiography and classified as presence (n=101) or absence (n=99) of severe (proliferative or preproliferative) DR. **Results:** 1) eNOS4: genotype distribution was eNOS4b/b 72%, eNOS4b/a 24.5% and eNOS4a/a 3.5%. Frequency of eNOS4a/a homozygous deletion was significantly different in patients with severe DR (0%) when compared with controls (7.1%, OR=0 [95%CI 0.5-0.74], p=0.02). eNOS4b/b was more frequent in patients with severe DR (79.2%) when compared with controls (64.6%, OR=2.1 [95%CI 1.1-4.12], p=0.032). Frequency of eNOS4b/a was not different between the study (20.8%) and control groups (28.2%, OR=0.7, p=0.2). The allelic frequencies between the study and control groups were different (4b:89.6 vs. 78.8%, OR=2.3 [95%CI 1.27-4.25], p=0.005; 4a:10.4 vs. 21.2%, OR=0.4 [95%CI 0.24-0.79], p=0.005). 2) VDR (Taq I): genotype distribution was TT 34.5%, Tt 51% and tt 14.5%. Frequency of TT was significantly lower in patients with severe DR (26.7%) when compared with controls (42.4%, OR=0.5 [95%CI 0.26-0.94], p=0.02). Frequencies of Tt,tt and its alleles were not different between the study and control groups (Tt:57.4 vs. 44.4%, OR=1.7, p=0.09; tt:15.8 vs. 13.1%, OR=1.3, p=0.7; T:55.4 vs. 64.6%, OR=0.7, p=0.07; t:44.6 vs. 35.4%, OR=1.5, p=0.07). **Conclusions:** we demonstrate for the first time, in type 1 diabetic patients, that 1) eNOS4a/a homozygous deletion is associated with low risk for severe DR, 2) eNOS4b/b is associated with high risk for severe DR, and 3) TT of Taq I polymorphism of the VDR is associated with low risk for severe DR.

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EXTRACELLULAR MATRIX GLYCATION IMPAIRS PERICYTE ADHESION AND REPLICATION.

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Background and Aims: Capillary pericytes contribute to synthesize the basement membrane together with endothelium and are selectively lost in diabetic retinopathy. We reported previously that pericytes from bovine retinal capillaries (BRP) seeded on extracellular matrix (ECM) obtained from HUVEC grown in high glucose (G) concentrations are less numerous than those grown on ECM produced in normal G. This study aimed at verifying the mechanisms of this finding. **Materials and Methods:** Conditioned ECMs were obtained by growing HUVEC in media with 5.6 or 28 mmol/l D-G, L-G or D-galactose (D-gal) up to 28 mmol/l, and 28 mmol/l D-G + 150 µmol/l thiamine (T) or 7 mmol/l aminoguanidine (AG). After 7 days, HUVEC were lysed and ECM fixed by NH4OH. BRP were cultured in normal G on these conditioned ECMs or on plates coated overnight with laminin, fibronectin and type IV collagen 10, 25 or 50 µg/ml. Cells were counted after 1 (to measure adhesion) or 7 days (replication). Cell proliferation (MTT method) and DNA synthesis (BrdU incorporation) were measured after 2 and 7 days. Results are expressed as percentages of BRP grown on ECM obtained in normal G. **Results:** BRP counts on ECM produced by HUVEC in high D-G (81.9±7.5% of control, p=0.000, day 1, 76.8±8.8%, p=0.000 day 7) and D-gal (87.3±9.5, p=0.004 day 1, 86.0±18.0, p=0.036 day 7) were lower than in normal D-G; L-G counts were lower at day 1 (90.7±9.1, p=0.015), but not at day 7 (96.4±31.3, NS). Both AG (103.6±8.4, p=0.000 vs high D-G, day 1, 96.6±14.7, p=0.003, day 7) and T (100.9±8.0, p=0.002 vs high D-G, day 1, 92.2±21.3, p=0.04 day 7) corrected this defect. MTT and BrdU assays showed a similar pattern. Type IV collagen in normal G did not modify BRP adhesion, while reducing counts at day 7 to 73.1±17.2% (p=0.012) at 10 µg/ml and 53.0±18.8% (p=0.002) at 50 µg/ml. In high D-G, at day 7, collagen further impaired BRP counts, both at 10 µg/ml (54.2±15.6% vs 71.0±8.0% in high D-G alone, p=0.001 vs normal G, p=0.021 vs high D-G) and 50 µg/ml (44.6±10.5, p=0.000 vs normal and high D-G). Fibronectin and laminin did not influence BRP counts at day 7 in either concentration, whilst at day 1 fibronectin in normal G increased BRP adhesion, both at 10 µg/ml (123.6±15.8%, p=0.015) and at 50 µg/ml (114.3±10.7, p=0.022). **Conclusions:** ECM produced by HUVEC in high D-G, L-G or D-gal is less supportive of BRP adhesion and replication. Excess protein glycation, corrected by AG and thiamine, may be involved, while overproduction of type IV collagen may contribute to impaired BRP replication. Such mechanisms might play a role in pericyte loss in diabetic retinopathy.

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High Glucose-Induced Connexin-43 Downregulation Inhibits Gap Junction Intercellular Communication in Rat Microvascular Endothelial Cells.

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Background and Aims: Connexins are gap junction proteins that are specialized membrane components involved in direct exchange of ions and small metabolites between cells allowing maintenance of tissue homeostasis. Since hyperglycemia results in disturbed vascular homeostasis, we investigated whether high glucose alters expression of gap junction protein, connexin-43 (Cx43), and affects gap junction intercellular communication (GJIC) activity.

Materials and Methods: In rat microvascular endothelial (RME) cells grown for 9 days in normal (5mM) or high (30mM) glucose medium, Cx43 localization, protein level and phosphorylation was analyzed using immunofluorescence microscopy, Western blot and immunoblot analysis after treatment of cell lysate with alkaline phosphatase. GJIC activity was determined by scrape load dye transfer (SLDT) technique.

Results: Immunofluorescence microscopy revealed Cx43 localization at sites of contact (plaques) between adjacent RME cells. Cells grown in high glucose medium exhibited reduced intensity of Cx43 immunofluorescence and reduced plaque count (63±6% of control, P=0.009) compared to cells grown in normal medium. Immunoblot analysis of RME cell lysates yielded three Cx43 forms corresponding to a nonphosphorylated form P0 (43kD) and two phosphorylated forms P1 and P2 (48, and 49kD, respectively). All three forms showed reduced expression under high glucose condition (73±15% of control, P=0.04; 57±16% of control, P=0.01; and 42±22% of control, P=0.006, respectively). The ability of cells to transfer Lucifer yellow through gap junctions was reduced under high glucose condition (3.9±0.6 vs 6.5±1.0, p<0.01, n=5). The reduced GJIC activity showed a strong correlation with the downregulation of Cx43 expression in high glucose cells (r=0.9).

Conclusions: Findings from this study indicate that high glucose-induced inhibition of Cx43 expression reduces GJIC activity in microvascular endothelial cells. Maintenance of the homeostatic balance through gap junction may be disturbed by high glucose condition resulting in endothelial cell dysfunction in diabetic microangiopathy.

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Benfotiamine prevents the consequences of hyperglycemia-induced mitochondrial overproduction of reactive oxygen species, and experimental diabetic retinopathy

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Background and Aims: Vascular complications are the main cause of morbidity and mortality in diabetes mellitus. Four seemingly independent biochemical pathways are involved in the pathogenesis: glucose-induced activation of protein kinase C (PKC) isoforms, increased formation of glucose-derived advanced glycation end products; increased glucose flux through the aldose reductase pathway, and increased flux through the hexosamine pathway. Hyperglycemia increases reactive oxygen species (ROS) production inside cultured bovine aortic endothelial cells. ROS activate aldose reductase, activate PKC, induce advanced glycation end product formation, activate the hexosamine pathway, and activate the pleiotropic transcription factor nuclear factor-kappa B (NFkB). The thiamine prodrug benfotiamine inhibits the formation of AGEs in target tissues of diabetic microangiopathy. **Materials and Methods:** Using bovine aortic endothelial cells, we studied the effect of benfotiamine on intracellular AGE-formation, flux through the hexosamine pathway, activation of protein kinase C, and activation of NFkB. Benfotiamine was added to cells in high glucose media at a final concentration of 50 µM. Media was changed daily for 7 days. AGE-formation was determined by dot blot technique, and complexes were visualized using an ECF kit (Amersham). Cell extracts were analysed on an HPLC system as described previously (PNAS 2000 97:12222-12226). For NFkB determination cells were incubated in low glucose, high glucose, high glucose + benfotiamine for 6 hrs. NFkB was determined by a fluorescence in situ DNA-protein binding assay and fluorescence /cell was determined using Scantalytics. PKC assay was performed after cells were incubated for 7 days as described above. The cells were analysed using a PKC assay system from Life Technologies. Additionally, diabetic rats (i.v. injection of streptozotocin 65 mg/kg body weight) were treated with benfotiamine (80 mg/kg weight) for 36 weeks, and diabetic retinopathy was assessed using quantitative retinal analysis of digest preparations for the development of acellular capillaries. Age-matched non-diabetic and untreated diabetic rats served as controls. **Results:** Benfotiamine decreases AGE formation by 60% using quantitative immunoblotting. UDP-GlcNAc was decreased 50% by benfotiamine using the previous conditions. NFkB was decreased 85% to below control levels. Benfotiamine decreased membrane PKC 55% to control levels. Chronic treatment of benfotiamine was well tolerated and did not result in significant changes of metabolic parameters. The development of acellular capillaries was reduced by benfotiamine-treatment from 72.5±11.16 acellular capillary segments/mm² of retinal area in diabetic rats to 29.64±4.48 acellular capillary segments/mm² in benfotiamine-treated rats (p<0.001). **Conclusion:** These data suggest that treatment with benfotiamine may be an effective approach to prevent the development of diabetic complications.

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THE ANGIOTENSIN TYPE-2 RECEPTOR IS ANTI-ANGIOGENIC AND DOWN-REGULATES VEGF AND VEGFR-2 EXPRESSION IN A RODENT MODEL OF RETINOPATHY OF PREMATURITY.

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Background and Aims: Both angiotensin II (ANG II) and vascular endothelial growth factor (VEGF) are angiogenic agents that have recently been implicated in the pathogenesis of proliferative diabetic retinopathy. Previously we reported that the angiotensin converting enzyme inhibitor (ACE) lisinopril and the angiotensin type 1 receptor (AT1) antagonist losartan, prevented retinal neovascularization in a rodent model of retinopathy of prematurity (ROP). Interestingly, although the cellular expression of vascular endothelial growth factor (VEGF) and its type 2 receptor (VEGFR-2) in retina were reduced in ROP rats treated with lisinopril (LIS), losartan (LOS) had no effect. These findings show that the retinoprotection and reduction in VEGF and VEGFR-2 expression afforded by ACE inhibition may involve the angiotensin type 2 (AT2) receptor. The current study aimed to determine if the AT2 receptor blockade (AT2-RB) influences retinal neovascularization and VEGF and VEGFR-2 expression in ROP. **Materials and Methods:** ROP was induced in newborn Sprague Dawley rats by exposure to 80% O₂ for 11 days followed by 7 days in room air. ROP shams were exposed to room air for 18 days from birth. Additional groups of ROP and ROP shams received the AT2-RB, PD123191, by miniosmotic pump (5mg/kg/day) from days 11-18 (retinal neovascularization period in ROP rats). The number of blood vessel profiles per histological section of inner retina (BVPs) was quantitated in at least 500 unit areas per eye. VEGF and VEGFR-2 were assessed using in situ hybridization. **Results:** In ROP rats treated with AT2-RB, BVPs were reduced to a similar extent as ROP rats treated with LIS or LOS (ROP untreated, 25.7 ± 1.5; AT2-RB, 14.9 ± 1.2; LIS, 17.3 ± 1.2; LOS, 19.4 ± 0.6, p<0.05). VEGF and VEGFR-2 mRNA in ROP retina were both reduced with AT2-RB (VEGF: ROP untreated, 59.2 ± 6.7; AT2-RB, 11.1 ± 1.7; LIS, 16.3 ± 1.7; LOS, 78.2 ± 9.2, p<0.05. VEGFR-2: ROP untreated, 54.0 ± 5.4; AT2-RB, 15.9 ± 2.8; LIS, 0.5 ± 0.5; LOS, 38.7 ± 8.3, p<0.05). **Conclusions:** These findings indicate that the anti-proliferative effects of renin-angiotensin system (RAS) blockade in the retina may involve down-regulation of VEGF and VEGFR-2 expression via the AT2 receptor. Blockade of the RAS may provide an important anti-angiogenic therapy for the treatment of a variety of ischemic-induced retinal pathologies.

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A NEW MODEL OF RETINAL AND IRIS NEOVASCULARIZATION IN THE DIABETIC TRANSGENIC (mREN-2)27 RAT: BLOCKADE WITH ACE INHIBITION.

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Background and Aims: New blood vessel growth in the retina and iris is common in patients with long-standing diabetes, and ultimately leads to visual impairment and blindness. The advent of new therapies for diabetic ocular neovascularization has been hampered by the lack of a diabetic rodent model that progresses to retinal and iris neovascularization. This study aimed to determine if the streptozotocin (STZ) induced diabetic transgenic Ren-2 rat which is hypertensive, displays an enhanced tissue renin-angiotensin system and develops advanced nephropathy, also exhibits retinal and iris neovascularization.

Materials and Methods: At 6 weeks of age, Ren-2, Spontaneously Hypertensive (SHR) and Sprague Dawley (SD) rats were randomised to receive either STZ (diabetic, D) or control vehicle (C). A separate group of diabetic Ren-2 rats were treated with the angiotensin converting enzyme (ACE) inhibitor lisinopril (L, 10mg/kg/day, drinking water) from the induction of diabetes. Rats were studied for 10 months. The number of proliferating endothelial cells (PEC; proliferating cell nuclear antigen and lectin immunohistochemistry) were quantified in 3 randomly chosen paraffin sections of retina and iris per eye. In situ hybridization was performed for vascular endothelial growth factor (VEGF) and its second receptor (VEGFR-2).

Results: SBP was higher in Ren-2 and SHR compared to SD rats (p<0.01) and was unchanged with diabetes. L reduced diabetic Ren-2 SBP (p<0.05). PECs in retina and iris were increased in untreated diabetic Ren-2 (retina Ren-2-C, 2.0 ± 0.9; Ren-2-D, 12.4 ± 6.9, p<0.05; iris Ren-2-C, 6.2 ± 2.6; Ren-2-D, 43.4 ± 12.1, p<0.05) and reduced with L (retina Ren-2-D+L, 0; iris Ren-2-D+L, 10.6 ± 1.5). In retina of non-diabetic and diabetic SHR and SD rats, PECs were not detected. PECs in irises of SHR and SD was unchanged with diabetes and decreased with L.

Conclusions: This study provides evidence of a new diabetic model of retinal and iris neovascularization that is associated with up-regulation of tissue renin and VEGF and VEGFR-2 gene expression. The anti-angiogenic effects of ACE inhibition supports our previous findings in a rodent model of retinopathy of prematurity and the EUCLID study which showed lisinopril to slow the progression to PDR in humans.

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Damage and Repair Mechanisms in Beta-Cells

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SURFACE ANTIGENS, HSP 70 AND SPECIFIC FUNCTION OF PANCREATIC ISLET BETA CELLS AFTER COMBINED ACTION OF CYTOKINES

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Background and Aims: During the development of type 1 diabetes the pancreatic islet beta cells are exposed to different cytokines which act not as single substances but in combination. Therefore, it is possible that different cytokines influence each other in the expression of antigens and/or in changing pancreatic islet beta cell function. The aim of the study was to investigate the influence of different cytokines and cytokine combinations on the expression of HSP 70, of the surface antigens MHC I and II and on the function of islet beta cells of diabetes-prone BB rats. **Methods:** Isolated pancreatic islets were precultured for 3 days and then exposed to IL-1β (10 U/ml) or IFN-γ (500 U/ml) or TNF-α (500 U/ml) or to combinations of cytokines: IFN-γ+TNF-α or IL-1β+IFN-γ+TNF-α. Untreated islets were used as controls. For the characterization of specific function insulin release and content were determined. Islet proteins were separated by SDS-PAGE followed by immunoblotting with the monoclonal antibody C92F3A-5 for HSP 70. Surface antigen expression was measured on single islet cells by FACS analysis using monoclonal antibodies OX18 (MHC I), OX6 (MHC II) and K14D10 (beta cells). **Results:** IL-1β alone induced the expression of HSP 70 but did not change the expression of MHC I or II on beta cells. Insulin content and release were diminished. These functional effects were not influenced by TNF-α and IFN-γ. IFN-γ alone had no effect on the expression of HSP 70 but induced MHC II on beta cells (7.8±1.8% vs. 1.9±0.5% in controls) and increased the MHC I antigen density (18.3±1.6% vs. 6.1±1.9 log U). The IFN-γ mediated increase of MHC II antigens on beta cells was enhanced by TNF-α (30.5±1.1% vs. 7.8±1.8%) whereas TNF-α alone had no effect on HSP 70 and on the expression of the surface antigens investigated. A combination of all three cytokines abolished completely the IFN-γ induced enhancement of MHC I antigen density. **Conclusions:** Our results demonstrate that the cytokines investigated influence each other in the expression of antigens on islet beta cells. The increased expression of an antigen induced by a single cytokine could be enhanced or even completely abolished by different combinations of cytokines. Thus, destruction or survival of islet beta cells may depend on the appearance of different cytokine combinations during an immune attack.

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Effects of selective binding to peripheral benzodiazepine receptors on the function and survival of human islet cells.

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Background and Aims: Peripheral benzodiazepine receptors (PBRs) are proteins mainly located on the mitochondrial membrane, where they are part of the mitochondrial permeability transition (PT) pore. PT pore regulates the function of mitochondria and therefore the survival of the whole cell. Following our demonstration that PBRs are present in human pancreatic islets (HI), now we describe the effects of a prolonged exposure of purified HI to PBRs ligands.

Materials and Methods: Two specific ligands, PK-11195 (PK) and RO 5-4864 (Ro), which have overlapping, but not identical binding domains and slightly different binding affinities were used. Human islets were exposed for 12h to the tested compounds, and islet function and survival were then evaluated.

Results: Glucose-stimulated (16.7 mM) insulin release (IR, μU/ml) was significantly lower after exposure to 0.5 μM PK (222±46, mean±SD, n=12) and 1.0 μM PK (160±71, n=10), than after exposure to 1.0 μM Ro (319±90, n=10), 10 μM Ro (341±88, n=10), or control medium (348±188, n=14). The amount of apoptotic cells was evaluated by the TUNEL technique (Ti) and the ELISA method, and was significantly higher in 1.0 μM PK-exposed HI (Ti: 42.3±7.9%, n=5; OD: 2.2±0.5, n=6) than in 10 μM Ro-exposed (Ti: 11.1±4.1%, n=4; OD: 1.24±0.2, n=4) and control (Ti: 14.3±6.6%, n=8; OD: 1.27±0.4, n=8) HI. Electron microscopy demonstrated typical apoptotic changes (cellular shrinkage, chromatin condensation, and apoptotic bodies) in PK-exposed human beta-cells. These effects were accompanied by no major change of mRNA expression of iNOS, Bax and Bcl 2, as evaluated by RT-PCR. Inhibition of up-stream caspases, caspase-3 or caspase-6 significantly reduced (30% or more) the amount of dead cells.

Conclusions: Thus, specific and selective binding to PBRs causes human beta-cells functional damage and apoptosis, a phenomenon which occurs without any clear change of iNOS, Bax and Bcl-2 mRNA expression, and involves caspase activation. These results raise the possibility that PBRs are involved in human pancreatic beta-cell function and survival.

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ESSENTIAL ROLE OF NF-kappaB (p50) IN THE DEVELOPMENT OF TYPE I DIABETES.

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Background and Aims: Development of Type I diabetes requires expression of immune-related genes such as MHC proteins, cytokines and cytotoxic enzymes, all regulated by transcription factors including NF-kappaB. The aim of this study was to determine if mice with a disruption of the NF-kappaB (p50) gene were protected from multiple-low-dose-streptozotocin (MLDS) induced diabetes. MLDS-induced diabetes is characterised by an immune cell-mediated destruction of the β -cells and a progressive hyperglycemia. **Materials and Methods:** Male NF-kappaB (p50)^{-/-} and NF-kappaB (p50)^{+/+} mice (n=17) were injected with stz (40mg/kg i.p.) or vehicle on five consecutive days, blood glucose was monitored over a 21-day period. Pancreas samples were taken on day 21 and insulin content determined. Islets were isolated from both phenotypes (n=4) and exposed to a combination IL-1 (100pM), TNF-alpha (100pM) and IFN-gamma (10U/ml) for 24h before glucose-stimulated insulin secretion was determined. Serum or culture media nitrite/nitrate levels were used as a measure of nitric oxide formation. **Results:** MLDS treated NF-kappaB (p50)^{+/+} mice progressively developed hyperglycemia, blood glucose levels 115±4 and 330±27mg/dl (p<0.01) on day 1 and 21 respectively, with 88% of the mice diabetic (blood glucose>200mg/dl). MLDS treated NF-kappaB (p50)^{-/-} mice had no incidence of diabetes and only a moderate increase in blood glucose levels from 111±3 to 139±3mg/dl (p<0.05). MLDS decreased pancreas insulin content in NF-kappaB (p50)^{+/+} mice from 61±11 to 17±3ng insulin/mg protein (p<0.01) an effect partially reversed in NF-kappaB (p50)^{-/-} mice; 54.8±8 to 31±4 (p<0.05). MLDS treatment increased serum nitrite/nitrate levels of NF-kappaB (p50)^{+/+} mice from 16±0.5 to 37±4μM (p<0.01), but not in the NF-kappaB (p50)^{-/-} mice; 25±3 to 26±4μM. In vitro cytokine treatment of NF-kappaB (p50)^{+/+} islets reduced insulin secretion from 2.6±0.4 to 1.2±0.2ng insulin/islet/h (p<0.05) and increased media nitrite/nitrate; 46±0.8 to 69±3.4pmol/islet/24h (p<0.05). However, despite a 50% reduction in nitric oxide formation; 39±2.6 to 49±2.54pmol/islet/24h (p<0.05) cytokines inhibited insulin secretion in NF-kappaB (p50)^{-/-} islets; 2.2±0.4 to 0.96±0.2ng insulin/islet/h (p<0.05). **Conclusions:** NF-kappaB (p50) plays a pivotal role in activation of the immune system and resulting nitric oxide formation during the development of type I diabetes. However, disruption of the NF-kappaB (p50) gene did not protect β -cells from cytokine-mediated induction of nitric oxide synthase and inhibition of insulin secretion. It appears that NF-kappaB (p50)'s involvement is at an early, critical juncture of immune-activation and pro-inflammatory mediator production.

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Investigation of cytokine-activated transcription factors in RINm5F cells overexpressing antioxidant enzymes by reporter gene and gel shift assays
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Background and Aims: Cytokines contribute to beta-cell damage in Type I diabetes mellitus and the intracellular destruction process is mediated by the induction of iNOS and other radical producing enzymes and their products NO and oxygen free radicals. During this destruction process through cytokines the activation of specific transcription factors, in particular NF-kB, plays a critical role. It was the aim of this study to characterise the importance of antioxidant enzymes and oxygen free radicals for the cytokine mediated activation of NF-kB in RINm5F cells.

Materials and Methods: Insulin-producing RINm5F cells overexpressing the antioxidant enzymes catalase (CAT), glutathione peroxidase (GPX), Cu/ZnSOD and MnSOD were transiently transfected with a NF-kB promoter element coupled to an alkaline phosphatase (SEAP) reporter gene. Cells were exposed to IL-1 β alone or to a cytokine mix. The accumulated reporter gene product SEAP was quantified at different time points. For gel shift Assays nuclear extracts from control and stimulated cells were isolated and incubated with radioactively labelled oligos with the NF-kB binding motif. DNA-protein complexes were resolved by a native PAGE and detected by autoradiography.

Results: The incubation of RINm5F control cells with IL-1 β or the cytokine mix results in a maximal 12fold increase of SEAP enzyme activities after 6 h. The activation of NF-kB in RINm5F-GPX and RINm5F-Cu/ZnSOD cells after incubation with IL-1 β was comparable with the activation in untransfected cells. In contrast the CAT overexpressing RINm5F cells showed a 15-20fold increase of the activation level. Overexpression of MnSOD caused a 80% decrease of NF-kB activation in comparison to untransfected RINm5F cells. Decreasing the expression of MnSOD by using the antisense technique resulted in an approximately 90% increase of NF-kB activation. Gel shift assays confirmed cytokine-mediated activation of NF-kB in control cells and in MnSOD overexpressing cells. In cells overexpressing MnSOD the activation of NF-kB was less in comparison with untransfected cells.

Conclusions: The activation of NF-kB after cytokine stimulation could be delayed or reduced by the overexpression of the antioxidant enzymes MnSOD and CAT in insulin-producing RINm5F cells. Thus, in addition to the direct inactivation of radicals cytoprotective enzymes are important modulators in the activation of transcription factors, in particular for the cytokine activated pathways of NF-kB. Importantly the mitochondrial inactivation of radicals affects the basal expression level of NF-kB.

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THE ANTIAPOTOTIC EFFECT OF THE JNK INHIBITOR TAT-JNK BINDING DOMAIN (JBD) IS INDEPENDENT OF IL-1 MEDIATED iNOS, ICE, Mn-SOD AND BCL-2 GENE EXPRESSION IN RIN CELLSM.A. Nikulina¹, N. Sandhu¹, Z. Shamshu¹, N.A. Andersen¹, A.E. Karlsen¹, C. Bonny² and T. Mandrup-Poulsen¹. ¹Steno Diabetes Center, 2820 Gentofte, Denmark and ²Division of Medical Genetics, CHUV, 1011 Lausanne, Switzerland

Background and Aims: The stress-activated protein kinase c-jun NH₂-terminal kinase (JNK) is a main signal for IL-1 β -induced apoptosis in insulin-producing cells, and the soluble JNK inhibitor Tat-JBD effectively prevents cytokine-induced β -cell apoptosis. The aim of this study was to investigate whether JNK inhibition affected the expression of pro- or antiapoptotic β -cell genes. **Materials and Methods:** RIN-5AH-T2B cells (750,000 cells/ml) were precultured for 24 h in RPMI 1640-Glutamax + 10% FBS + 1% Pen/Strep at 37°C prior to incubation for 1 h with 1μM Tat-JBD and followed by addition of 60 U/ml of rIL-1 β . After 6 and 24 hours the total RNA was isolated, cDNA was generated by the cDNA Cycle Kit (Invitrogen) and RT-PCR was performed using Tata-binding protein or β -glucuronidase as housekeeping genes to normalize gene expression. Accumulated insulin release to the media was measured by ELISA and nitrite production by means of the Griess reagent. Hoechst 33342 and propidium iodide nuclear staining were used for apoptotic counts after 48 hours of exposure to 400 U/ml of rIL-1 β . **Results:** Tat-JBD reduced JNK activity by 35% (p < 0.001) and caused a 55% inhibition of IL-induced apoptosis (p < 0.001). However, Tat-JBD did not influence IL-1 β -induced NO synthesis (3.0±0.54 μM vs. 3.4±0.39 μM, n=12) or iNOS expression and only slightly decreased cytokine-mediated inhibition of insulin release at 24 h (18.7 ± 1.17 nM vs. 22.1±1.28 nM, n=12, p < 0.01). Further, Tat-JBD did not prevent the IL-1-induced increase in Bcl-2, Mn-superoxide dismutase (Mn-SOD), IL-1 β -converting enzyme (ICE), or caspase-3 gene expression. **Conclusion:** The antiapoptotic effect of JNK was independent of the transcription of major pro- and antiapoptotic genes, but may be exerted at the level of translation or enzyme activity. Primary targets for JNK-mediated β -cell apoptosis still remain to be identified.

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Suppressors of cytokine signaling (SOCS) in cytokine-induced human islet cell damage

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Background and aims: Type I diabetes results from the selective destruction of islet beta-cells through mechanisms not fully elucidated. Beta-cell damage is due to infiltrating T-cells, macrophages and inflammatory cytokines (IFN γ , TNF α , IL-1 β). In addition, in animal models, cytokines such as IL-4 and IL-10 can be protective, being able to counteract the adverse effects of pro-inflammatory cytokines, reducing beta-cell damage. SOCS belong to a protein family that negatively regulate cytokine signaling. SOCS genes are induced by a variety of cytokines and SOCS proteins induced by one can inhibit signaling by another (e.g. IL-10 can suppress expression of IFN γ -induced genes in human monocytes through SOCS-3). Previously we have shown that: 1) Human islets treated, in vitro, with IFN γ +TNF α +IL-1 showed a significant decrease of glucose-stimulated insulin release (IR). 2) Islets pre-cultured with IL-4 and IL-10 followed by IFN γ +TNF α +IL-1 showed a glucose-stimulated IR similar to untreated islets. 3) Preincubation with IL-4 and IL-10 significantly reduced the rate of islet cell death induced by IFN γ +TNF α +IL-1. Consequently, aims of our study were to investigate, at islet cell level, on the expression of SOCS genes, their inducibility by cytokines and on their possible involvement in protective effects of IL-4 and IL-10.

Materials and Methods: mRNA expression of SOCS-1, -2 and -3 genes was evaluated in isolated human islets, cultured for 1h and 10h, in the presence of: 1) IL-4, IL-10, IFN γ , TNF α and IL-1, separately; 2) a cocktail of IFN γ +TNF α +IL-1; 3) IL-10 followed by a cocktail of IFN γ +TNF α +IL-1. RNA expression of SOCS molecules was analyzed by RT-PCR, followed by quantitative scanning densitometry.

Results: 1) SOCS-1, -2 and -3 mRNA is expressed in untreated human islets; 2) SOCS-1 mRNA expression is upregulated at 10 hours by proinflammatory cytokines; 3) SOCS-2 mRNA expression is upregulated by IL-4, by IL-10 and by TNF α within 1 hour and its expression remains high up to 10 hours; 4) SOCS-3 mRNA expression is upregulated after 10 hours by all cytokines tested.

Conclusions: SOCS-1, -2 and -3 are indeed expressed in human pancreatic islets, where they can be up-regulated by treatment with cytokines, thus representing a potential pathway of modulation of cytokine action. Early upregulation of SOCS-2 expression, induced by IL-4 and IL-10, suggests a possible role of this molecule in the modulation of the protective effects of these two cytokines on human islets when challenged with inflammatory cytokines.

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Clinical Aspects of Diabetic Pregnancy

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IS THERE ANY CUTOFF POINT OF HbA1c LEVELS INDICATIVE OF THE NEED FOR INSULIN TREATMENT IN WOMEN WITH GESTATIONAL DIABETES?

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Background and Aims: It is known that HbA1c levels are not useful in the diagnosis of gestational diabetes mellitus (GDM). However there are no data whether HbA1c levels may help to determine therapeutic strategy for GDM women. The aim of this study is to define if there are any cutoff points of initial HbA1c levels, which may identify the subgroup of GDM women for whom insulin therapy will be needed, according to their pre-pregnancy BMI and trimester. **Materials and Methods:** Based on ADA 2000 GDM criteria, 2144 pregnant women had a normal OGTT- 100g and 1079 where diagnosed as GDM. From the GDM group 424 where treated with insulin in addition to diet therapy. Criteria for insulin initiation where at least one of the following: (a) fasting plasma glucose >95mg/dl; (b) one hour post-prandial glucose >140 mg/dl; (c) fetal macrosomia by ultrasound. BMI was calculated according to pre-pregnancy body weight in all pregnant women, who where divided in three groups, based on BMI: (i) Normal weight group (BMI-1) 18.5-24.9 kg/m², n=1826; (ii) Overweight (BMI-2) 25.0-29.9, n=883; (iii) Obese (BMI-3) >30, n=514. In all pregnant women HbA1c levels were measured (HPLC- Menarini) at the time the OGTT was performed. The total number of HbA1c measurements were 1658 in the second and 1565 in the third trimester. The optimum HbA1c insulin therapy-indicative cutoff points and their sensitivity and specificity where calculated according to BMI group and trimester of pregnancy. **Results:** The HbA1c cutoff points determined, according to BMI group, in the second trimester were: (i) BMI-1: HbA1c >4%, Sensitivity: 65%, Specificity: 64%; (ii) BMI-2: HbA1c >4.1%, Sensitivity 65%, Specificity 67%; (iii) BMI-3: HbA1c >4.3%, Sensitivity 72%, Specificity 75%. In the third trimester the figures were (i) BMI-1: HbA1c >4.1%, Sensitivity 69%, Specificity 67%; (ii) BMI-2: HbA1c >4.4%, Sensitivity: 79%, Specificity 77%; (iii) BMI-3: HbA1c >4.5%, Sensitivity 75%, Specificity 77%. **Conclusions:** Initial HbA1c levels, though not used as a diagnostic criterion for GDM, may be useful in the identification of the subgroup of GDM women, especially the obese ones, who will need insulin therapy.

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IS PREGNANCY A RISK FACTOR FOR MICROVASCULAR COMPLICATIONS? THE EURODIAB PROSPECTIVE STUDY. O. Verier-Mine*, N. Chaturvedi**, D.J. Webb**, A. Quigley*, D. Bensoussan* and J.H. Fuller** on behalf of the EURODIAB IDDM Complication Study Group. *CH Valenciennes France. **Dept.Epid.Publ.Health, University College of London, UK.

Background and aims: Controversy exists on long term influence of pregnancy on development and progression of IDDM microvascular complications. **Materials and methods:** in the EURODIAB prospective multicentre study, 793 women potentially child bearing at baseline completed the follow-up (7.3 years) and 163 (21%) gave birth. We compared risk factors (RF) (mean levels of age, duration of diabetes, HbA1c, and proportion of giving birth) for developing a microvascular complication between progressors and non-progressors. **Results:** A) For women childless at baseline giving birth in follow-up (102/425): 1) 32/267 progressed to microalbuminuria. High HbA1c was a RF. Age, duration of diabetes or giving birth [25% vs 23%, p=0.8, adjusted Odd Ratio (OR) for significant co-variables 1.32(0.55,3.22)] were not RF. 2) 43/282 progressed to proliferative retinopathy. Duration of diabetes and high HbA1c were RF, whereas giving birth was not [26% vs 25%, p=0.9, adjusted OR 1.57(0.65,3.79)]. For progression to any form of retinopathy (104/186), again duration of diabetes and HbA1c were RF but not giving birth [20% vs 30%, p=0.1, adjusted OR 0.70(0.32,1.51)]. 3) For neuropathy, 59/288 progressed. Giving birth was not a RF: 25 % vs 25%, p=0.9, [OR 1.03 (0.53,1.99)]. B) For women with children at baseline giving birth in follow-up (61/368): 1) 35/235 progressed to microalbuminuria; high HbA1c was a RF but giving birth was not: 9% vs 17%, p=0.2, adjusted OR 0.56(0.15,2.05). 2) 23/257 progressed to proliferative retinopathy. Giving birth was not a RF: 13% vs 16% p=0.7, adjusted OR 1.30(0.34, 4.91). 82/166 progressed to any retinopathy; giving birth was not a RF: 12% vs 23%, p=0.08, adjusted OR 0.50(0.21,1.22). In both cases, duration of diabetes and high HbA1c were RF. 3) 59/244 progressed to neuropathy: age was a RF, not giving birth: 12% vs 21%, p=0.1, adjusted OR 0.69(0.27,1.74). **Conclusions:** In this European study, having a first or another pregnancy did not seem to be a risk factor for progression of any microvascular complication. This is in accordance to the findings of the American DCCT Study

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A NATION-WIDE PROSPECTIVE STUDY ON THE OUTCOME OF PREGNANCIES IN WOMEN WITH TYPE 1 DIABETES MELLITUS; DO PLANNED PREGNANCIES RESULT IN BETTER PREGNANCY OUTCOME?

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Background and Aims: Periconceptional glycaemic control has been shown to be a major determinant for the occurrence of congenital malformations (CM) in the offspring of women with type 1 diabetes mellitus (DM1). Motivating type 1 diabetic women to plan their pregnancy, resulting in good glycaemic control and folic acid use periconceptionally, will hopefully optimise pregnancy outcome. We conducted a nation-wide prospective study to assess periconceptional preparation and glycaemic control as well as the frequency of CM, perinatal (PM) and maternal mortality (MM).

Materials and Methods: A total of 364 women with DM1 were included within 1 year from 103 hospitals in the Netherlands; 14 women had an abortion, 15 had type 2 diabetes or secondary diabetes and in 20 there were incomplete data, leaving 315 subjects (mean age 30 (SD 4) years, mean duration of disease 13 (SD 8) years and 98% Caucasian). Periconceptional glycaemic control was assessed using HbA1c-levels determined at about 10 weeks gestation in one central laboratory (normal non-diabetic HbA1c-range 4.0-6.0%). CM were divided into major CM, like cardiac anomalies, neural tube defects and genitourinary system abnormalities and minor CM, like hypospadias, vertebral anomalies and single umbilical artery.

Results: Eighty-four percent of the women reported that they planned their pregnancy. Seventy percent of the women started the use of folic acid before conception. Mean HbA1c-level early in pregnancy was 6.7% (SD 0.8%) (range 4.4-9.0%); 72% of the HbA1c-levels was good (<7.0%). The overall CM-rate was 7.9% (normal population rate 2.0%, p<0.05). Major CM occurred in 4.8% and minor CM in 3.1%. HbA1c-levels were significantly higher in pregnancies with CM compared to those without CM (7.2% (SD 0.9%) vs. 6.6% (SD 0.7%), p<0.05). The occurrence of major CM was significantly higher in unplanned pregnancies compared to planned pregnancies (10.0% vs. 3.4%, p<0.05). PM was 3.2% (normal population rate 0.9%, p<0.05). MM was 0.6% (n=2) (normal population rate 0.01%, p<0.05). Causes of maternal death were severe hypoglycaemia and amnion fluid embolism.

Conclusions: Despite a high frequency of planned pregnancies, resulting in a high rate of adequate folic acid use and overall good glycaemic control early in pregnancy, congenital malformations, perinatal and maternal mortality were still strongly increased. This indicates that in current conditions pregnancy in type 1 diabetic women remains a high-risk situation. Further reducing unplanned pregnancies to improve pregnancy outcome seems an important goal to achieve.

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Pre-eclampsia associated with altered post-partum insulin resistance and production in non-diabetic subjects

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Background and Aims: Pre-eclampsia (PREC) has been associated with fetal morbidity like intra-uterine growth restriction and fetal death as well as with maternal complications. Endothelial cell dysfunction is thought to be pivotal in the pathogenesis of PREC. Since insulin resistance (IR) is also related to endothelial cell dysfunction, PREC may be a clinical entity to which IR contributes. We assessed IR and beta-cell function (BCF) in non-diabetic women after a pregnancy with PREC.

Materials and Methods: A total of 213 women after a pregnancy complicated by PREC were studied: mean age: 31.2 (SD: 4.5 years), median time since childbirth 0.4 years (95th percentile (p95): 3.58); 70% were studied within 6 months. Fasting plasma glucose and insulin were determined; glucose was maximally 6.1 mmol/l to include normoglycaemic subjects only. IR was determined using the formula: $\ln(\text{insulin}/22.5 \cdot e^{-\ln(\text{glucose})})$, BCF by $(20 \cdot \text{insulin})/(\text{glucose} - 3.5)$. The subjects were divided into subjects giving birth before week 34 (early (E): age: 30.7 (SD: 4.5)) and after week 34 (late (L): age: 32.6 (SD: 4.5)). Normal values for IR and BCF were derived from 43 women (C; age: 32.9 (SD: 4.5)).

Results: Significant differences were observed in IR, BCF and age between the groups (Kruskal-Wallis, p<0.001). IR was higher in E than in L: 2.45 (p95: 6.97) vs 1.73 (5.41), p<0.001 and than in C: 1.88 (5.31) p=0.007. IR in L and C were similar. BCF was higher in E than in L: 154 (p95: 417) vs 120 (363), p=0.03 and than in C: 94 (515) p<0.001 with the difference between L and C of borderline significance (p=0.049). With ANOVA to correct for difference in age, IR in E was higher than in L (p=0.004) and C (p=0.001) with a significant difference in BCF between L and C (p=0.004). No differences in IR or BCF between L and C were found. IR and BCF were positively associated (r=0.65, p<0.001), also after correction for age (r=0.54, p<0.001), also in E, L, and C separately. Duration of pregnancy at childbirth was inversely associated with IR and BCF (r=-0.21, p<0.007 and r=-0.17, p=0.03). Duration of pregnancy at diagnosis of PREC was also inversely associated with IR and BCF (r=-0.26, p=0.003 and r=-0.21, p=0.02), both suggesting more severe abnormalities with more severe PREC.

Conclusions: Pre-eclampsia leading to childbirth before week 34 is associated with higher insulin resistance and insulin production post-partum, suggesting that pre-eclampsia is associated with altered insulin dynamics. Since these phenomena were studied post partum, further studies during pregnancy are indicated.

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Gestational Diabetes Mellitus: predictive factors for the development of diabetes at mid-term follow-up.

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Background and Aims: Women with gestational diabetes mellitus (GDM) have a high risk of developing diabetes (DM) later in life and several predictors have been described. The aim of this study was to identify antepartum and postpartum predictive factors for the development of DM at mid-term follow-up in a group of women with prior GDM.

Materials and Methods: In 696 women with prior GDM, a 75g-OGTT was performed postpartum (median 6.16 years, range 0.05, 13.73) which was assessed according to WHO-1998 criteria. A Cox multiple hazard regression analysis was used to ascertain if the following variables were independent predictors: previous glucose intolerance, family history of diabetes, parity, history of poor obstetric outcome, prepregnancy body mass index (BMI), age at pregnancy, gestational age at GDM diagnosis, GDM diagnostic OGTT (glycemic values, area under the curve and number of abnormal values), autoantibody positivity (islet cell, glutamic acid decarboxylase and tyrosine phosphatase antibodies), insulin treatment during pregnancy, neonatal weight > 4000, preterm delivery, recurrence of GDM and age and BMI increment at follow-up.

Results: Forty four women (6.3%) developed DM with a cumulative risk of 13.7% at 11 years of follow-up. Previous glucose intolerance (RR 6.73, $p=0.003$) and the higher quintile of prepregnancy BMI (BMI > 26.4 RR 8.67, $p=0.001$) were independent predictors of developing DM. The 3rd tertile of the number of abnormal values at GDM diagnosis (4 abnormal values/clinical diagnosis) had borderline significance (RR 4.78, $p=0.054$). Overall, these factors provided a sensitivity of 67%, specificity of 76%, positive predictive value of 14.9% and negative predictive value of 97.3% for DM prediction.

Conclusions: In these women with GDM, prepregnancy glucose intolerance and high BMI are independent risk factors for DM at follow-up.

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CARDIOVASCULAR AND METABOLIC ABNORMALITIES IN OFFSPRING OF PRE GESTATIONAL TYPE 1 DIABETIC PREGNANCY

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Background and Aims The concept that maternal fuel metabolism may exert long range effects on the offspring of diabetic pregnancy was first proposed by Jorgen Pedersen over 20 years ago. Long-term follow up studies among ethnic groups with high rates of type 2 diabetes have demonstrated a high prevalence of glucose intolerance and obesity in the offspring of diabetic pregnancy. Few studies have examined whether similar outcomes pertain among offspring of pre gestational type 1 diabetic pregnancy or whether the impact of the intrauterine diabetic milieu might extend to other cardiovascular risk factors besides glucose and body weight. The aim of this study was to examine markers of cardiovascular disease in childhood among offspring of type 1 diabetic pregnancy.

Materials and Methods 61 pre-pubertal offspring aged 5-11 years (26 male/35 female) of pre gestational type 1 diabetic pregnancies were randomly selected from maternity records and compared with 57 offspring of non diabetic subjects matched for age, sex and social class. Each child underwent measurement of height (m), weight (kg), triceps and subscapular skin fold thickness (mm) and blood pressure (Omron). Fasting blood was obtained for glucose, insulin, IGF-1, plasminogen-inhibitor 1, fibrinogen, leptin (DSL, ELISA), lipid profile, and the adhesion molecules VCAM-1 and E-selectin. Insulin resistance was calculated using the HOMA model. Data was analysed using SPSS version 10.

Results Skin fold thickness, blood pressure, glucose, insulin, insulin resistance and leptin did not differ between the two groups. Significant differences between the mean values for diabetic versus non diabetic offspring were: total cholesterol (4.45 vs 4.18, $p=0.025$); LDL cholesterol (2.73 vs 2.39, $p=0.001$); cholesterol/HDL ratio (3.42 vs 3.09, $p=0.027$); IGF1 (22.49 vs 19.30, $p=0.036$); PAI-1 (19.95 vs 14.87, $p=0.000$); VCAM (1845.59 vs 1508.93, $p=0.000$) and E-selectin (88.66 vs 71.12, $p=0.010$). **Conclusions** These data support the Pedersen hypothesis and indicate that metabolic and cardiovascular risk factors exist in glucose tolerant offspring of diabetic pregnancy aged 5-11 years compared with non diabetic offspring. The findings have implications for the management of diabetic pregnancy and suggest the need for long term follow up of these subjects.

OP 28

Glucose Transport

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The Novel Glucose Transporter GLUT10 is Expressed in Human Skeletal Muscle Cells

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Background and aims: The facilitative hexose transporters (GLUT-family) are membrane proteins which differ in their tissue distribution, kinetic characteristics and substrate specificity. Human GLUT10 is a novel sugar transporter-like gene which exhibits significant sequence similarity with the members of the GLUT family. In human tissues, a 7.2 kb transcript of GLUT10 was exclusively detected in heart and skeletal muscle. The aim of this study was to examine the regulation of GLUT10 expression in human skeletal muscle cells during differentiation and different metabolic situations, and to assess the insulin-sensitivity of this novel transporter.

Materials and Methods: Primary human skeletal muscle cells were obtained as proliferating myoblasts, and were fused to myotubes for different time periods. GLUT10 expression was determined by Northern and Western blotting; cell surface biotinylation was used to study the translocation of GLUT10 in response to insulin.

Results: In myoblasts, GLUT10 was detected as a 40 kDa protein. Upon differentiation the abundance of GLUT10 increased about 3fold reaching a stable expression by 8-12 days. This was confirmed by a comparable increase in GLUT10 mRNA. Increased expression of GLUT10 in myotubes was paralleled by a 3-4fold increase in the expression of the insulin receptor. Expression of GLUT10 was not affected by the presence of high glucose (25 mM) in the culture medium. The presence of fructose (25 mM) reduced GLUT10 abundance by $40 \pm 3\%$ ($n=4$); the same effect was observed after glucose starvation (overnight) of the myotubes. Biotinylation of myotubes before and after stimulation with insulin (10 min) indicated an increased abundance of GLUT10 at the cell surface in response to the hormone.

Conclusion: GLUT10 represents a novel glucose transporter that is expressed in differentiated human skeletal muscle. Our data suggest that the overall abundance of GLUT10 is subject to metabolic regulation, and that GLUT10 is redistributed to the plasma membrane in response to insulin.

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RABEF4: A POSSIBLE LINK BETWEEN INSULIN SIGNALING AND GLUT4 TRAFFIC?

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Background and Aims: The small GTPase Rab4 is involved in the regulation of Glut 4 trafficking. To better understand its role in insulin action, we searched for Rab4 effectors by screening a fat mouse cDNA library. We cloned a cDNA coding for a protein of 609 amino acids that interacts with active Rab4 in yeast. This protein, named Rabef4 (Rab4 effector), contains three SH3 domains, two proline-rich regions and a coiled-coil domain and is ubiquitously expressed. The specific aim of this study has been to characterise the role of Rabef4 in insulin-sensitive cells.

Materials and Methods: A Tet-Off stable cell line of 3T3-L1 fibroblasts overexpressing myc-Rabef4 was selected in which Rabef4 expression was obtained in the absence of tetracycline either in fibroblasts or adipocytes.

Results: In Tet-Off fibroblasts and adipocytes insulin induces a rapid and stable association of myc-Rabef4 with endogenous Rab4. A specific inhibitor of PI3K, wortmannin, was able to prevent the formation of Rabef4-Rab4 complex, suggesting an important role of the PI3K pathway in this association. Endogenous or overexpressed Rabef4 is coprecipitated with tyrosine phosphorylated proteins after insulin treatment of both fibroblasts and adipocytes. Further, while a PDGF treatment induces the association of myc-Rabef4 with tyrosine phosphorylated proteins in 3T3-L1 fibroblasts, it has no effect in adipocytes. One of the tyrosine phosphorylated proteins that coprecipitates with Rabef4 is a 120 kDa protein. We tested whether it corresponds to Cbl, a protein that is tyrosine phosphorylated in response to insulin in adipocytes. By coimmunoprecipitation, we demonstrate that Cbl and Rabef4 are indeed associated. Further, insulin induces the delocalization of Rabef4 from a Triton X100 soluble to a Triton X100 insoluble fraction, as it does for Cbl.

Conclusions: The formation of a complex of Rabef4, Cbl and possibly Rab4 associated to the lipid rafts may lead to the generation of specific signals important for the regulation of glucose transport. Rabef4 would thus appear as a possible link between insulin signalling and Glut 4 traffic.

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NELFINAVIR, AN HIV PROTEASE INHIBITOR, IMPAIRS INSULIN SIGNALING & GLUT4 TRANSLOCATION IN 3T3-L1 ADIPOCYTES

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Background and Aims: HIV protease inhibitors are potent anti-retroviral agents clinically used in the management of HIV infection. Recently HPI therapy has been linked to the development of a metabolic syndrome, in which adipocyte insulin resistance appears to play a major role.

Materials and Methods: Fully differentiated 3T3-L1 adipocytes were exposed for 18 h to up to 40 microM nelfinavir.

Results: Nelfinavir treatment resulted in impaired insulin stimulated glucose uptake (ISGT) with an EC50 of approximately 20 mM. The reduction in ISGT could be attributed to impaired GLUT4 translocation to the plasma membrane, while total membrane GLUT4 content was unaltered. Insulin stimulated insulin receptor and insulin receptor substrate phosphorylation, and the association between IRS1 and the p85 unit of PI 3-kinase, were not affected by nelfinavir even at concentrations which induced impaired glucose uptake. However, nelfinavir treatment severely impaired the insulin stimulation of both Ser473 as well as Thr308 PKB phosphorylation, with no effect on total PKB content. Interestingly, insulin stimulated ERK1/2 and p70S6 kinase phosphorylation were also decreased following nelfinavir treatment. Troglitazone pre and co-treatment increased ISGT by 20% in control, but not in nelfinavir treated cells, and could not protect against the reduction in insulin effect.

Conclusions: These data may provide a cellular explanation for the peripheral insulin resistance and abnormal adipocyte function in treated HIV patients.

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Essential Role of the SUMO-Conjugating Enzyme Ubc9 for Insulin-Stimulated Glucose Transport in Adipocytes.

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Background and Aims: We have recently shown that the GLUT4 glucose transporter interacts with the SUMO-conjugating enzyme Ubc9 and is covalently conjugated with SUMO-1. The objective of this study was to investigate the relevance of Ubc9 to insulin regulation of glucose transport in adipocytes. **Materials and Methods:** The Ubc9 protein was transiently reduced in 3T3-L1 adipocytes by treating cells with a specific phosphorothioate antisense oligonucleotide for 72 h or overexpressed by adenovirus-mediated Ubc9 gene transfer. **Results:** Ubc9 protein levels were reduced 80% in adipocytes treated with the Ubc9 antisense as compared to cells left untreated or treated with sense, reverse, or scrambled oligonucleotide sequences. The decrease in Ubc9 was associated with a 70% reduction in GLUT4 protein levels compared to control ($P < 0.05$). By contrast, the levels of the GLUT1 transporter were unchanged. In adipocytes treated with the Ubc9 antisense basal glucose transport rates were not different than control, but insulin-stimulated glucose transport was markedly reduced by 75% ($P < 0.05$). Inhibition of Ubc9 biosynthesis did not modify the expression of various insulin signaling proteins, such as the insulin receptor, IRS-1, IRS-2, PI 3-kinase, and Akt, nor it affected insulin stimulation of IRS tyrosine phosphorylation or Akt activity. On the other hand, infection of adipocytes with a recombinant adenovirus encoding the Ubc9 cDNA increased Ubc9 protein levels 4-fold and resulted in a 2.5-fold increase in total GLUT4 levels as compared to cells transduced with the control gene beta-Galactosidase ($P < 0.05$). Ubc9 overexpression was associated with no change in basal glucose transport, but significant enhancement of the fold-stimulation of glucose transport by insulin (8.0-fold vs. 4.2-fold, $P < 0.05$). GLUT4 mRNA levels were not modified by Ubc9, indicating that GLUT4 regulation by this enzyme does not involve changes in GLUT4 gene transcription. **Conclusions:** the SUMO-conjugating enzyme Ubc9 plays a critical role in regulating the cellular levels of GLUT4 in adipocytes post-transcriptionally. Therefore, Ubc9-mediated SUMO conjugation represents a novel mechanism for physiological regulation and pharmacological targeting of insulin-stimulated glucose uptake in insulin-sensitive cells.

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Defective P2Y purinergic receptor function induces impaired glucose transport in type 2 diabetes

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Background and Aims: P2Y receptors (P2YR) are membrane-spanning G-protein-coupled receptors whose activation by extracellular ATP (eATP) triggers generation of inositol 1,4,5-triphosphate and calcium mobilization from intracellular stores. They are involved in regulating platelet aggregation, cytokine release, smooth muscle cells contraction. Recently, a role of P2YR in modulating glucose transport (GT) in rat cardiomyocytes has been suggested. Aim of this study was to evaluate the effect of P2YR stimulation on glucose uptake (GU) in skin fibroblasts from 6 healthy subjects (C) and 6 type 2 diabetic patients (T2D). **Materials and Methods:** P2YR expression was evaluated by RT-PCR; changes in intracellular calcium concentration by the fluorescent indicator Fura2/AM; GLUT1 was identified in three membrane fractions by immunoblotting and chemiluminescence; GU was measured with the analogue 2-deoxy-D-[1-3H] glucose (2-DOG) and eATP in the medium by luminometry with the luciferin-luciferase assay. **Results:** 2-DOG uptake from T2D was basically insensitive to eATP stimulation (basal 100 ± 12 in C and 85 ± 15 pmol/mg/min in T2D; with ATP 1 mM 230 ± 21 in C and 126 ± 13 pmol/mg/min in T2D). GLUT1 was equally expressed in C and T2D; its content in the Golgi, however, was much lower in T2D. eATP was able to promote GLUT1 association to the plasma membrane in both cells, but this event per se was not sufficient to drive an increased GU in T2D. Expression of P2YR did not differ in the two groups. Intracellular calcium release, a functional response typically triggered by P2YR, was reduced in T2D respect to C (102 vs 248 nM). Pretreatment with hexokinase, an ATP hydrolysing enzyme, completely restored P2YR-mediated calcium release in T2D (228 vs 217 nM in C), suggesting a desensitized state of this receptors in T2D. This hypothesis was confirmed by a three-fold higher ATP content in the supernatants of T2D compared to C (0.2 ± 0.5 and 0.08 ± 0.2 micrograms ATP/1,000,000 cells). Intracellular ATP, otherwise, did not differ between the two cell populations. Accordingly, incubation in the presence of hexokinase re-established ATP-dependent GU (140 ± 10 in C and 156 ± 12 pmol/mg/min in T2D). **Conclusions:** extracellular ATP appears to modulate insulin-independent GT, P2YR-dependent GLUT1 activation being deficient in type 2 diabetes. These observations suggest possible additional targets for improving glucose utilization in diabetes.

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DECREASED CONTENT OF IRS-1, IRS-2 AND GLUT4 IN RAT ADIPOCYTES WHEN EXPOSED TO HIGH GLUCOSE AND INSULIN

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Background and aims: The pathogenesis of type 2 diabetes involves complex interactions among multiple physiological defects. The purpose of this study was to investigate the cellular effects of long-term exposure to high insulin and glucose on glucose transport and insulin signalling proteins in primary cultured rat adipocytes.

Materials and methods: Isolated adipocytes were obtained by collagenase treatment of epididymal fat from Sprague-Dawley rats. The cells were cultured for 24 h in DMEM containing 5, 10, 15 or 25 mM D-glucose in the presence or absence of insulin (10,000 uU/ml). After washing, basal and insulin-stimulated 14C-D-glucose uptake was performed and total cell lysates or membranes were prepared. Insulin signalling peptides were assessed by immunoblotting.

Results: Long-term incubation with high glucose (10, 15 and 25 mM) for 24 h induced a dose-dependent decrease in basal and insulin stimulated glucose uptake compared to control cells incubated in 5 mM glucose. High glucose (25 mM) down-regulated IRS-1 expression by ~50%, whereas IRS-2 was strongly up-regulated by glucose levels of 10 mM glucose or more (by ~200-600%). Neither glucose nor insulin affected PI3-K and PKB levels. Surprisingly, 15mM glucose increased GLUT4 of cellular membranes (by ~100%, $p < 0.001$) compared to 5mM. 24 h insulin treatment had a negative effect on glucose uptake only in the presence of high glucose (by ~30-60% at 15 mM glucose, $p < 0.001$). High levels of insulin in the incubation medium almost abolished IRS-2 expression and amplified the suppression of IRS-1 produced by 15 or 25 mM glucose. Insulin also decreased GLUT4 at high glucose (by ~60%, $p < 0.001$).

Conclusions: Long-term exposure to high glucose per se can reduce glucose transport capacity independent of GLUT4 expression. Concomitant incubation with high insulin markedly impairs the expression of IRS-1, IRS-2 and GLUT4 and this may contribute to insulin resistance, e.g. in type 2 diabetes.

OP 29

Searching for the Artificial Beta-Cell

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FIRST IMPLANTATIONS OF A LONG TERM GLUCOSE SENSOR CONNECTED TO INSULIN PUMPS IN DIABETIC HUMANS

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Background and Aims: Implanted insulin pumps have been used in diabetic subjects for more than 10 years. Connecting an intra-vascular (IV) glucose sensor to the pump would provide real-time data to enable regulation of insulin delivery, leading to a true 'artificial beta-cell'. The aim of the study is to assess the safety and accuracy of an implanted sensor/pump system. **Methods:** Long term glucose sensor systems (LTSS, Medical Research Group and MiniMed Inc., CA, USA), consisting of an IV glucose sensor connected to a pump for intra-peritoneal (IP) insulin delivery, were surgically implanted into ten type 1 diabetic subjects for approximately 18 patient-months. Sensors were placed through subclavian or internal jugular veins, with sensor tips in the superior vena cava or right atrium. Sensor data were compared to six daily capillary blood glucose sample readings on HemoCue photometers (HemoCue AB, Sweden). **Results:** Surgical complications consisted of placing and maintaining the sensor tip in the proper location. Optimization of the surgical procedure and modification of the connecting cable facilitated attachment and prevention of migration. A total of 3788 paired points yields a Mean Absolute Difference (MAD) of 19.5% over the range of 1.5 to 21.5 mmol/L, a correlation coefficient (r) = 0.90, and 97% of paired points within the A & B ranges of the Clarke Grid. These data were sustained after 5 months of implant. **Conclusions:** Our data suggest that the LTSS can perform to sufficient clinical accuracy over an extended period of time to control IP insulin delivery.

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HYPO- AND HYPER-GLYCEMIC EXPOSURE ESTIMATES BASED ON CONTINUOUS GLUCOSE SENSOR DATA PREDICT GLYCOSYLATED HEMOGLOBIN.

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Background and Aims: HbA1c is the most widely accepted measure of glucose control, as it increases in direct proportion to glycemic exposure over the previous 90 days. The Continuous Glucose Monitoring System (CGMS, MiniMed Inc.) gives a complete picture of glucose levels over 3 days of use and provides direct evidence of the duration and magnitude of glucose excursions above and below desired limits. The present analysis was conducted to determine if the integrated area under the curve (AUC), derived from a brief period of CGMS use, can predict long-term glucose control, as reflected in HbA1c.

Materials and Methods: A historical HbA1c value was obtained from 238 patients (198 type 1, 40 type 2) at 13 clinical centers, who then used the CGMS for between 1 and 18 days (Mean = 4.2 days). The incremental area of the glucose curve above 10 mmol/L (AUC-HI) and below 3.9 mmol/L (AUC-LO) were calculated directly from the CGMS 5-minute readings, adjusted for the duration of sensor use and expressed as mmol*hr/L. Multiple linear regression and correlation analysis was performed using AUC-HI and AUC-LO as predictors of HbA1c.

Results: Subjects were (Mean + SD) 35.6 + 16.8 yrs. old, 57% female, with a 15.4 + 10.7 yr. history of diabetes, 55% using CSII and 31% using MDI. Subjects' hyperglycemic exposure (AUC-HI, 30.4 + 29.2 mmol*hr/L) was positively correlated with HbA1c ($r = 0.56$, $p < 0.0001$) and their hypoglycemic exposure (AUC-LO, 3.6 + 3.6 mmol*hr/L) was negatively correlated ($r = -0.28$, $p < 0.0001$). Regression analysis confirmed that both AUC-HI and AUC-LO were significant predictors of HbA1c (RSQUARED=0.32, $p < 0.0001$) with a prediction equation of $HbA1c = 7.1\% + (0.03)AUC-HI (0.06)AUC-LO$.

Conclusions: These results demonstrate that a brief period of continuous glucose sensor data can provide detailed information on the duration and magnitude of both low and high glucose excursions. Indices based on these excursions can be used to predict long-term glucose control. These estimates can provide immediate feedback on treatment intervention and will be useful in treatment decisions, patient education and broader research applications.

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Evaluation of a Global Modeling Approach for Non-Invasive Blood Glucose Measurements

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Background and Aims:

Previously, we demonstrated that it is possible to accurately measure blood glucose values non-invasively using near-infrared spectroscopy for extended periods of time, provided that the device has been calibrated to the individual. The aim of this study is to determine if a global approach to calibration can be achieved, thereby reducing the regimen required for calibrating the individual to the device.

Materials and Methods:

Over 60 adult diabetic subjects who utilize intensive insulin management are being studied during the course of this trial. Non-invasive near-infrared data and corresponding blood glucose values (HemoCue) were collected during a series of modified glucose tolerance tests. A variety of proprietary multivariate modeling techniques were used to develop and evaluate the global modeling approach.

Results:

To date, the global modeling approach which we have employed has lead to accurate results being achieved in 40 of the 60 subjects studied. The duration of the global modeling approach has varied from 1 to 8 weeks. Evaluation of these measured values based on the Clark Error Grid yields approximately 55% of the data in the A region, 39% of the data in the B region, 3% in each of the C and D regions, with no data falling in the E region. The absolute error is approximately 22%.

Conclusions:

For the first time, there is evidence that it is possible to develop a general model for non-invasive blood glucose measurement that accurately measures blood glucose levels on independent test data. These results motivate the continued development of a home-use non-invasive blood glucose monitor based on near-infrared spectroscopy.

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DEVELOPMENT OF CLOSED-LOOP INTRAPERITONEAL INSULIN INFUSION ALGORITHM

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Background and Aims: To apply artificial endocrine pancreas (AEP) to diabetic patients for longer-period, we need to develop the implantable type (implantable AEP). In order to achieve the physiological insulin delivery route, intraperitoneal insulin infusion may be important. For this purpose, we have developed the closed-loop intraperitoneal insulin infusion algorithm.

Materials and Methods: 1) By analyzing the kinetics of intraperitoneal absorption of insulin, we have developed intraperitoneal insulin infusion algorithm. The intraperitoneal insulin infusion rate was calculated according to Eq. $IIR(t) = Kp \cdot G(t) + Kd \cdot dG(t)/dt + Kc$; Kp, Kd, Kc: insulin infusion parameters 2) Insulin infusion patterns and plasma insulin profiles were obtained from simulation study. Furthermore, we apply this algorithm to five alloxan induced diabetic dogs, and control blood glucose concentration after oral glucose load.

Results: 1) Insulin infusion parameters were set as $Kp=0.041$, $Kd=2.91$ and $Kc=-2.72$. 2) Using this algorithm, the plasma insulin level was simulated to be normal after oral glucose load, and was similar to that using intravenous infusion algorithm. By applying this algorithm, near-physiological glycemic control was achieved without showing any delayed hyperinsulinemia or hypoglycemia.

Conclusions: These results indicate that the application of intraperitoneal insulin infusion algorithm is feasible for long-term glycemic control with AEP.

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Correlation of Standard Measures of Glucose Control with A New Optical Method of Glycemic Assessment in Patients At Risk for Type 2 Diabetes
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Background and Aims: Current measures of glycemic assessment require a blood sample. A new noninvasive method for assessing glycemic control status, Accu-Chek™ D-Tector™ (ACDT), has been developed based on detection of glycated proteins in the lens of the eye using a blue laser and measuring the resultant fluorescence. This innovative technology may expand diabetes screening to nontraditional settings (e.g., professional eye care), increasing access to the undiagnosed population. We present preliminary results from a 2-year study correlating standard measures of glycemic assessment (FPG, HbA1c; fructosamine) with the results of the ACDT.

Materials and Methods: Approximately 250 patients identified as at risk for type 2 diabetes by the Community Diabetes Prevention Project (those with a + FH of diabetes and at least 1 component of the insulin resistance syndrome, but not yet having IGT) are being tested every 6 months using standard glycemic assessment tests and ACDT.

Results: ACDT tests compared with FPG diagnosis had a specificity of 88.7% (yielding 24 additional positive results among a population of 212 previously negative patients) and a sensitivity of 45.0% (matching 5 out of 11 positive results by FPG criteria; sensitivity = 50% with the exclusion of one patient who had been told he had diabetes and started treatment prior to ACDT testing). For the "false positives" the salient question is whether this new technology has provided early identification of patients who have abnormal glucose homeostasis but have not yet developed diabetes by FPG criteria. Longitudinal data will reveal how effectively the ACDT detects abnormalities in overall glycemic control. Abnormal lens glycation may identify patients with diabetes or indicate elevations in overall glycemic exposure, signaling possible impending diabetes (since even elevations of plasma glucose in the normal range have been correlated with future development of diabetes).

Conclusions: The noninvasive Accu-Chek™ D-Tector™ optical test for glycemic assessment represents a promising new approach for the early detection of diabetes, and may help to meet the need for ongoing systematic screening in expanded clinical settings.

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Glucose monitoring in the adipose tissue of type 1 diabetic patients using open-flow microperfusion and microdialysis

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Background and Aims: The objective of this study twofold: first to compare two methods for glucose sampling in the adipose tissue: open-flow microperfusion (OFM), and microdialysis (MDial), and second to determine local differences of the glucose concentration in the adipose tissue of type 1 diabetic patients.

Materials and Methods: Both sampling techniques are based on perfusing a probe with isotonic, ionfree mannitol (0.5 µl/min). With OFM the interstitial fluid (ISF) enters the OFM catheter through macroscopic perforations. With MDial partial equilibration of the perfusate with the ISF occurs through the dialysis membrane of the MDial probe. In order to determine the degree of exchange of the fluids (recovery) the concentration of sodium was measured in the recollected perfusate (ionic reference technique). One OFM catheter and one MDial probe were inserted into the adipose tissue of 6 patients with IDDM (BMI 27.0 ± 2.5 kg·m⁻², age 43.2 ± 7.4 years) at least 3 cm apart from each other. For 9 hours ISF samples were collected in 30 min intervals with corresponding plasma values. During the investigation the patients followed their usual meal - insulin pattern.

Results: The plasma glucose concentration ranged between 3.47 and 17.83 mmol/l (n=92). The correlation coefficient of linear regression analysis of plasma glucose and glucose sampled with OFM / MDial was between 0.57 and 0.95 / 0.65 and 0.96, respectively. The mean difference of the glucose concentration sampled simultaneously with OFM and MDial in the same subject was 1.46 ± 0.18 mmol/l. Taking the different recoveries of the two catheters into account and applying the ionic reference technique the correlation was considerably increased for OFM (0.92-0.99) and MDial (0.96-0.99). Furthermore, the absolute difference in simultaneously measured adipose tissue glucose concentration was reduced to 0.05 ± 0.10 mmol/l.

Conclusions: Although there is a large difference of the glucose concentration between the catheters due to different properties of the techniques (membrane, size, exchange area) this difference can be taken into account with the ionic reference technique. It can be concluded that there is no difference between OFM and MDial regarding their capability to determine ISF glucose concentration. There are also no local differences in glucose concentrations in the adipose tissue. Therefore both techniques as well as the site of sampling (adipose tissue) seem to be appropriate for glucose monitoring.

OP 30

Determinants of Cardiovascular Disease in Diabetes

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GLYCOHEMOGLOBIN VALUES OVER 18 YEARS PREDICT CORONARY ATHEROMATOSIS IN TYPE 1 DIABETICS.

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Background and Aims: Diabetes type 1 patients have a pronounced risk of premature development of coronary artery disease. Intracoronary ultrasound examination gives detailed information on atherosclerotic plaques. The standard method for evaluation of coronary artery disease, quantitative coronary angiography, is not sufficiently sensitive to detect non-symptomatic coronary artery disease. We evaluated the prevalence of preclinical atherosclerosis with intra coronary ultrasound and related the findings to HbA1c measured prospectively over 18 years. **Materials and Methods:** In 1982 45 type 1 diabetes patients were randomized to intensive insulin treatment with pump (n=15) or multi-injections (n=15) or to stay on 2-injections daily. After 4 years having identified the beneficial effects of intensive insulin treatment on microvascular complications, intensive therapy was advised to all. 39 patients are still in the study, 2 are dead (breast cancer, chronic lung disease) and 4 are lost to follow up. 29 Patients were examined with intra coronary ultrasound and quantitative coronary angiography. Mean HbA1c over 18 years was 8.2% (6.6-11.3) (normal 4.1-6.4). The mean duration of diabetes was 30 years (23-39). Mean age of the patients was 43 years (33-58). **Results:** All patients examined with intracoronary ultrasound in the present study had developed plaques. Linear regression shows a significant relation between degree of plaque formation and mean HbA1c value over 18 years when adjusted for age. (p=0.013). With intracoronary angiography 34% have more than 50% stenosis in 1 or more of the coronary arteries. There was no significant relationship between these findings and mean HbA1c over 18 years. **Conclusions:** Our study shows that asymptomatic coronary atherosclerosis is very frequent in type 1 diabetes of long duration and that mean value of HbA1c over 18 years predicts the degree of coronary atherosclerosis.

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PULSE PRESSURE PREDICTS THE RISK OF CARDIOVASCULAR EVENTS IN A LARGE COHORT OF PATIENTS WITH TYPE 2 DIABETES

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Background and Aims: Pulse pressure is a major determinant of cardiovascular risk in normotensive and hypertensive subjects. Increased pulse pressure reflects the arterial stiffening that occurs at an early stage in diabetes. The aim of this study was to investigate the relationship between pulse pressure and cardiovascular risk in patients with Type 2 diabetes.

Materials and Methods: Data on 2911 cases were abstracted from the Cardiff Diabetes Database describing a wide range of risk factors and events. The baseline year was 1996 with follow-up for four years. Parametric and non-parametric multivariate regression techniques were used to investigate for associations. Of the 2911 cases, there were 574 CHD events, 168 cerebrovascular disease (CVD) events, and 157 peripheral vascular disease (PVD) events.

Results: The various mathematical techniques produced generally consistent results. Using univariate analysis, after adjustment for age, sex, and duration of diabetes, there was an association between systolic BP and CHD (p=0.005), as well as pulse pressure and CHD (p=0.004). Both systolic BP and pulse pressure were associated with CVD (p<0.05). Pulse pressure was more strongly associated with PVD than was systolic BP (p<0.01 and p<0.05, respectively). After adjustment, total cholesterol (TC) and LDL cholesterol were associated with SBP (p<0.001 and p<0.05, respectively). Recursive partitioning identified TC and HDL cholesterol as the most important variables associated with pulse pressure after age.

Conclusions: This study demonstrates an association between CVD, PVD and CHD with a surrogate measure of increased arterial stiffness. Pulse pressure may be a more appropriate and sensitive clinical outcome measure for the assessment of hypertension in people with Type 2 diabetes.

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1,5-Anhydro-D-glucitol (AG) - marker of glucose excursions - in patients with advanced coronary heart disease.

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Objective: Decreased serum 1,5-Anhydro-D-glucitol (AG) has been reported in diabetic humans. The reduction of AG has been sensitively and specifically demonstrated in diabetes mellitus as an effect of impaired glycaemic control within 2-3 days preceding estimation. The data are consistent with the hypothesis that daily excursions of glycaemia play a pathogenic role in the advance of coronary heart disease. Recently unrecognized subtle changes in daily glycaemia predispose the heart to failure, after ischaemia-induced remodeling, and arteriosclerotic plaques to instability and rupture. These changes act in conjunction with effects, driven by hyperglycaemia and diabetes, on the endothelium of large blood vessels, e.g. on nitric oxide release or on protein kinase-C β activation. **Design and Methods:** 31 patients with coronary heart disease were involved into the study. The metabolic control parameters - fasting glycaemia, glycated hemoglobin A_{1c} and the new short-term marker of hyperglycaemia - anhydroglucitol (AG), cholesterolaemia, triglyceridaemia were estimated. Additionally fasting serum concentration of proinsulin, insulin, C-peptide were estimated. Proinsulin, insulin, C-peptide level and HbA_{1c} were determined using ELISA. Anhydroglucitol level was estimated enzymatically by Yaabuchi method in our own modification.

Results: Metabolic markers values were respectively: cholesterolaemia, triglyceridaemia insignificantly increased, fasting glycaemia, glycated hemoglobin A_{1c} - were within normal range, while AG level was significantly decreased [12.9 ± 5.8 mg/l] comparing with references value. Fasting serum proinsulin concentration was within normal range, C-peptide level insignificantly increased and insulin level increased. **Conclusions:** Meticulous glucose control early on and rapid recompensation of hyperglycaemia in patients with coronary syndrome are a part of successful intensive multifactorial approach to prevent the heart converting from ailing to failing. The routine used parameters of glycaemic control (fasting glycaemia, glycated hemoglobin A_{1c}) are often insufficient for complete characteristic of daily glycaemia. The serum 1,5-Anhydro-D-glucitol concentration may be useful as an indicator of glycaemic control in this patients.

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Myocardial inotropic function is related to insulin sensitivity in type 2 diabetic patients

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Background and Aims: In type 2 diabetic patients, non-ischaemic left ventricular (LV) dysfunction is more prevalent than in non-diabetic subjects. LV dysfunction may result from the metabolic alterations of diabetes. We tested whether in type 2 diabetes insulin resistance is associated with a worsening of LV inotropic function.

Materials and Methods: Insulin sensitivity was measured by means of the euglycaemic hyperinsulinaemic (40 mU/min/sqm) clamp technique. LV inotropic function was measured by means of two validated echocardiographic techniques: LV midwall fractional shortening (MFS) normalised to end-systolic wall stress (MFS/ESS, in percent) and by cyclic variation in backscatter ultrasound signal (CV, in decibel). Twenty-nine male and 4 female diabetic patients with no evidence of previous myocardial infarction and with normal LV chamber function endocardial fractional shortening = $40 \pm 2\%$ on standard echocardiography were studied. None was taking inotropic drugs. Nine patients had arterial hypertension and were treated with β -blockers (n=2), ACE inhibitors (n=6) and diuretics (n=1). Study group mean age was 58 ± 2 yrs, duration of disease 7 ± 1 yrs, BMI 28.4 ± 0.7 kg/m², HbA_{1c} $7.2 \pm 0.2\%$, and LV mass index 55 ± 6 g/m².

Results: Insulin-stimulated glucose uptake (M) averaged (mean \pm SEM) 34 ± 2 μ mol/min per kg of fat-free mass, MFS/ESS was $109 \pm 2\%$, and CV 6.0 ± 0.2 dB. When patients were divided into tertiles of M (21 ± 1 , 31 ± 1 and 50 ± 3 μ mol/min per kg of fat-free mass), the mean tertile values of MFS/ESS and CV were 105 ± 3 , 110 ± 3 , $114 \pm 4\%$ (p=ns by ANOVA) and 5.5 ± 0.3 , 5.8 ± 0.3 , 6.7 ± 0.3 dB (p<0.03 by ANOVA), respectively. In univariate analysis, M was correlated with both MFS/ESS and CV (r=0.50, p<0.01 and r=0.64, p<0.0001, respectively). The association between MFS/ESS and M was unchanged (partial r=0.52) after adjustment for age, sex, BMI, hypertension (or anti-hypertensive treatment), duration of diabetes, HbA_{1c} and LV mass index. In univariate analysis, CV was reciprocally related to diastolic blood pressure (r=-0.34, p<0.05) and BMI (r=-0.45, p<0.01). In multivariate analysis, only M remained independently associated with CV (partial r=0.51, p<0.01).

Conclusions: In type 2 diabetic patients with normal left ventricular chamber function, insulin resistance is associated with impaired LV inotropic function.

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IMPAIRED CORONARY FLOW RESERVE IN PATIENTS WITH TYPE 2 DM OR IMPAIRED FASTING GLUCOSE

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Background and Aims: Diabetes mellitus is associated with vasomotor dysfunction. However, it is not known whether both the prediabetic state of impaired fasting glucose (IFG) and type 2 DM reduce coronary flow reserve (CFR), and whether this is related to the lipid profile of these patients.

Materials and Methods: We measured myocardial blood flow in 12 middle-aged patients with dietary treated type 2 DM, 12 patients with IFG and 8 age-matched healthy controls with positron emission tomography (PET) using ¹⁵O-water at rest and during dipyridamole induced hyperaemia. CFR was calculated as the ratio of hyperaemic flow to resting blood flow.

Results: When compared to the control group, CFR was 37% lower in the type 2 DM group (2.7 ± 1.1 vs 4.2 ± 1.1 , p<0.01) and 23% lower in the IFG group (3.3 ± 1.1 vs 4.2 ± 1.1 , p<0.05). An inverse correlation was found between glycaemic control as measured by fasting plasma glucose (p<0.02) or HbA_{1c} (p<0.01) and CFR. Total cholesterol (TC) and LDL cholesterol levels were slightly lower in the type 2 DM and IFG groups compared to the control group, and the HDL/TC ratio did not differ between the groups. Triglyceride levels were higher in the type 2 DM and IFG groups compared to healthy controls. After adjusting for triglycerides, the difference in CFR remained significant between the type 2 DM and control groups (p<0.05).

Conclusions: Both type 2 DM and IFG impair coronary flow reserve. This impairment is related to glycaemic control, but it is not directly related to the lipid profile of these patients.

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A randomised double-blind placebo-controlled trial of a low dose continuous combined Hormone replacement therapy in women with type 2 diabetes

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Background and Aims: Data on the effects of oral oestradiol alone in women with diabetes are encouraging with evidence of improved insulin resistance and glycaemic control. However, oral oestradiol alone can increase C-reactive protein (CRP), a marker of low-grade chronic inflammation, that is already elevated in diabetes as well as promoting pro-coagulants effects (increase FVII), thereby leading to increased early CHD risk as in HERS. Further, most women require a combined preparation for endometrial protection but data on the metabolic effects of such preparation in diabetes are currently absent. We performed a randomised double-blind placebo-controlled trial of a low dose continuous combined HRT (1mg 17-beta oestradiol and 0.5mg norethisterone acetate) and speculated that this preparation would not adversely influence metabolic control and may lessen likelihood of increase in CRP.

Materials and Methods: 45 women were recruited and randomised to active treatment or matching placebo. Blood samples were taken at baseline and at 6 months for lipids, clotting, inflammatory and endothelial markers. In addition, we assessed glycaemic control and fasting insulin as a surrogate of insulin resistance. Serum hormones were also taken to confirm compliance with active drug or placebo. The women had mean ages of 60.4 and 60.2 yrs, BMIs of 30.3 and 30.5 kg/m² and HbA_{1c} of 10.1 and 10.3%, in the active and placebo groups, respectively (P=NS). All categories of diabetes therapy were included (diet, oral hypoglycaemic and insulin).

Results: There were no detectable changes in glycaemic control nor in cholesterol, triglyceride or HDL-cholesterol. However, fasting insulin decreased by 15% in the active group (P=0.043 relative to change in placebo group), sensitive IL-6 decreased by 5% (P=0.027), factor VII decreased by 14% (P<0.001), tPA decreased by 12% (P=0.023) and VCAM-I decreased by 23% (P=0.049), all MWU for comparison of change in active group versus change in placebo group. Further, CRP did not increase with active treatment. **Conclusions:** The low-dose continuous combined HRT preparation used in this study has overall beneficial effects in women with type 2 diabetes with significant improvements in several pathways related to vascular disease including vascular function, clotting, inflammation and perhaps also insulin resistance. Accordingly, future studies should address the potential for this preparation to reduce risk of macrovascular disease in women with type 2 diabetes.

OP 31

Molecular Insulin Resistance

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OXIDATIVE STRESS INDUCES PROTEIN DEGRADATION OF IRS1 THROUGH A PI-3K DEPENDENT, UBIQUITIN INDEPENDENT PATHWAY
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Background and Aims: Exposure to oxidative stress has been shown to induce insulin resistance, as well as decreased IRS1 content. The present study was conducted to investigate the mechanisms by which oxidative stress induces IRS1 protein degradation.

Materials and Methods: Differentiated 3T3-L1 adipocytes or the hepatoma cell line FAO cells were exposed for 2 h to 30 μ M H₂O₂ generated by adding glucose oxidase to the culture medium.

Results: Oxidative stress resulted in both cell types in decreased IRS1, but not IRS2 content, an effect which could not be altered by the presence of cycloheximide, suggesting protein degradation as the major mechanism. Concomitantly, increased serine phosphorylation was induced by oxidative stress, as assessed by both anti-pSer antibodies and reversal of the gel shift retardation in Western blot following alkaline phosphatase treatment. While increased total protein ubiquitination (assessed by Western blot) could be documented in oxidized cells, the proteasome inhibitor lactacystin did not inhibit the reduction in IRS1 content, while dramatically preventing the decrease in IRS1 content which was induced by chronic exposure to insulin. Pre-treatment with GSH ethyl ester increased cellular GSH by approximately 3-fold, but did not protect against neither IRS1 degradation nor the impairment in insulin stimulated glucose uptake. Both the PI 3K inhibitor LY294002 and the mTOR inhibitor rapamycin prevented both the degradation of IRS1 as well as its gel retardation. Yet, the MEK1 inhibitor PD98059 had no similar effect.

Conclusions: Exposure of adipocytes and hepatoma cells to H₂O₂ results in increased total protein ubiquitination, but inhibiting the proteasome system does not prevent IRS1 degradation. Both the degradation of IRS1 induced by oxidative stress, as well as its increased Ser phosphorylation, are PI 3K dependent.

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INCREASED EXPRESSION OF PROTEIN KINASE C- θ IN MUSCLE OF PATIENTS WITH TYPE 2 DIABETES FOLLOWING A GLUCOSE LOAD

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Background and Aims: In rodent models of insulin resistance, increased activity of protein kinase C (PKC), especially the calcium-independent isoforms (eg PKC- θ), phosphorylate and down-regulate the insulin receptor and key insulin signaling enzymes, but there are no clinical studies investigating PKC pathways in human skeletal muscle.

Methods: Male patients with type 2 diabetes (T2DM, n=7) and age-matched non-diabetic controls (n=8) attended the clinical research unit on two occasions, 1-week apart, for (1) measurement of insulin sensitivity using the euglycaemic hyperinsulinaemic clamp; and (2) a muscle biopsy (vastus lateralis) 2h after an oral 75g glucose challenge. RT-PCR analysis was undertaken to semi-quantitatively assess expression of PKC- θ mRNA, relative to GAPDH, in diabetic and control subjects.

Results: T2DM subjects were insulin resistant relative to controls ($M = 3.0 \pm 0.4$ vs 8.6 ± 0.8 mg/kg/min) and skeletal muscle expression of PKC- θ mRNA was 7-fold higher in patients with diabetes ($0.63\% \pm 0.25$ of GAPDH mRNA) compared with controls ($0.09\% \pm 0.07\%$ of GAPDH mRNA, $p=0.03$), but there was only a weak inverse correlation with in vivo insulin sensitivity.

Conclusions: Increased expression of PKC- θ mRNA in skeletal muscle of patients with T2DM confirms animal data showing that glucose-induced PKC activation is likely to contribute insulin resistance in diabetes. PKC- θ may mediate the link between muscle triglyceride content and insulin resistance.

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Transgenic Mice with Dominant Negative PKC-theta in Skeletal Muscle: a New Model of Insulin Resistance and Obesity

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Background and Aims: PKC-theta is a novel member of the PKCs superfamily that is specifically expressed in skeletal muscle (and hematopoietic cells) but not in other insulin-sensitive tissues (e.g., adipose tissue). Its expression, intracellular localization, and activity in the skeletal muscle is insulin-regulated, and correlates with insulin action, while its actual role in the regulation of insulin sensitivity is still not defined.

Materials and Methods: We produced transgenic (TG) mice that express a dominant negative mutant form of PKC-theta (the kinase-dead mutant form K/R) under the control of the skeletal muscle specific enhancer of the desmin promoter, therefore with the activity of PKC-theta specifically inhibited in all skeletal muscle fibers. TG mice were compared with age matched control wild type control mice (C), housed in identical cages and fed ad libitum with the same standard chow.

Results: When examined at 4 months of age, TG mice displayed normal weight (~ 30 g), a slightly increased (but not statistically significant) fasting insulin concentration (0.20 ± 0.03 vs. 0.13 ± 0.03 ng/ml, TG and C respectively), fed insulin and comparable glucose tolerance (i.p. glucose tolerance test), either in terms of glucose or insulin concentrations. Insulin tolerance test (i.p. insulin), however, showed reduced insulin sensitivity in TG mice. After the fourth month of age, TG mice started to rapidly gain weight, reaching, at 11-13 months, a mean weight of 56 ± 1.5 g (35 ± 1.7 g in age matched C, $p < 0.001$), with macroscopically evident accumulation of fat both subcutaneously and in visceral depots. At this age, moderate to severe hyperinsulinemia developed in TG mice (fasting: 0.47 ± 0.08 vs. 0.26 ± 0.03 ng/ml, $p < 0.05$; fed: 5.5 ± 1.0 vs. 2.2 ± 0.8 ng/ml, $p < 0.03$; 30' after ipGTT 1.20 ± 0.37 vs. 0.50 ± 0.05 ng/ml, $p < 0.05$) but still near to normal glucose tolerance (plasma glucose 30' after ipGTT 371 ± 33 vs. 324 ± 50 mg/dl, $p = NS$).

Conclusions: These data strongly suggest that PKC-theta is not only regulated by the insulin signal cascade, but also that its expression and activity profoundly affects insulin sensitivity. Finally, this new TG model suggests that a mildly reduced insulin sensitivity, experimentally induced only in skeletal muscle, might cause modest hyperinsulinemia which in turn diverts nutrients toward other insulin-sensitive tissues (adipose tissue), with the final outcome of severe obesity and (partially secondary) severe insulin-resistance. A similar mechanism (skeletal muscle less insulin sensitive than adipose tissue) might be proposed in human obesity and, finally, in diabetes mellitus.

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Long-term administration of AICAR reduces metabolic disturbances and lowers blood pressure level in animals displaying features of the insulin resistance syndrome
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Background and Aims: The insulin resistance syndrome (IRS) is characterized by several established or potential risk factors for cardiovascular disease. Chronic chemical activation of the AMP-activated protein kinase by the adenosine analog AICAR has been shown to augment insulin action, to up-regulate mitochondrial enzymes in skeletal muscles and to decrease the content of intraabdominal fat. Furthermore, acute AICAR exposure has been found to reduce sterol- and fatty acid synthesis in hepatocytes. With background in the effects of chronic AICAR administration on muscle metabolism and abdominal fat together with the possible influence of AICAR on liver metabolism, the present study was undertaken to explore, whether long-term AICAR administration was capable of improving major disorders associated with IRS.

Material and Methods: Obese Zucker (AICAR) rats were daily subcutaneously injected with AICAR (0.5 mg/g body weight) for 7 weeks. Obese controls were either pair-fed (PF) or fed ad libitum (AL). Lean (Lean) Zucker rats served as a reference group. Circulating levels of glucose, insulin, lipid and blood pressure were measured before and after 7 weeks of treatment. Furthermore an oral glucose tolerance test (OGTT) was performed after 7 weeks of treatment and finally the post-treatment level of retroperitoneal and epididymal fat was determined to evaluate potential changes in fat content.

Results: AICAR-administration normalized the oral glucose tolerance test and decreased fasting concentrations of glucose (5.95 ± 0.11 mmol/l) and insulin (282 ± 56 pmol/l) close to the level of the Lean-animals (glucose: 6.05 ± 0.30 mmol/l and insulin: 125 ± 12 pmol/l), obese controls had significantly higher values (AL animals: glucose 7.35 ± 0.44 mmol/l and insulin: 1119 ± 94 pmol/l and PF animals (glucose 7.25 ± 0.44 mmol/l and insulin: 1209 ± 200 pmol/l)). In addition, AICAR administration significantly reduced plasma triglycerides and free fatty acids, and increased HDL-cholesterol levels. Furthermore, AICAR-treatment lowered systolic blood pressure and finally, AICAR treated animals exhibited a tendency towards decreased intraabdominal fat content.

Content: Our data provide strong evidence that long-term administration of AICAR improves glucose tolerance, the lipid profile and reduces systolic blood pressure in an insulin resistant animal model. We propose the hypothesis that AMPK activation might be a possible future pharmacological strategy for treatment of features of the insulin resistance syndrome.

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MECHANISM OF AMINO ACID INDUCED INSULIN RESISTANCE IN HUMANS

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Background and Aims: Protein-rich diet may decrease insulin sensitivity (Linn et al, Diabetologia, 2000). Thus, this study was designed to determine the effect of short term elevation of plasma amino acids (AA) on whole body glucose uptake and cellular mechanisms of insulin action in skeletal muscle.

Materials and methods: Seven healthy men were studied for 5.5 h during euglycemic [5 mmol/l]-hyperinsulinemic [430 pmol/l]-basal glucagon [55 ng/l]-somatostatin clamp tests in the presence of basal (~1.5 mmol/l) and increased (~4.0 mmol/l) infusion of a balanced mixture of 21 AA plasma AA concentrations. To examine the metabolic pathways by which AA induce insulin resistance in humans the rate of muscle glycogen synthesis and muscular glucose-6-phosphate (G6P) concentrations were determined by ¹³C- and ³¹P-nuclear magnetic resonance spectroscopy, respectively.

Results: A 2.6-fold elevation in plasma AA reduced rates of whole body glucose uptake by ~25 % from 90 min on (P<0.01). Rates of muscle glycogen synthesis decreased to 38 % of control values between 180 and 330 min (28±7 vs. control: 69±8 µmol/[kg·min]; P<0.01). Reduction of muscle glycogen synthesis by elevated plasma amino acids was preceded by a decrease in muscle G6P concentrations starting at ~130 min (ΔG6P_{260-300 min}: 18±19 vs. control: 103±33 µmol/l; p<0.05).

Conclusions: Plasma amino acid elevation induces skeletal muscle insulin resistance in humans by inhibition of glucose transport/phosphorylation resulting in marked reduction of muscle glycogen synthesis.

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Sympathetic Blockade increases Insulin-induced Vasodilatation and Glucose Uptake

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Background and Aims: Insulin infusion causes both vasodilatation in skeletal muscle as well as sympathetic stimulation. The insulin-induced increase in sympathetic outflow may compensate and in part offset insulin-induced vasodilatation. Acute sympathetic activation induces insulin resistance. We hypothesised that blockade of sympathetic nervous outflow may unmask a stronger vasodilator effect of insulin. Because insulin-induced vasodilatation may support insulin-induced glucose uptake, blockade of the sympathetic nervous system may thus have a favourable effect on insulin sensitivity.

Materials and Methods: Fourteen normal subjects were studied. Group 1 (n=6) underwent repeated graded NicotinicN-receptor blockade with intravenous trimethaphan (TRI). In the first test, autonomic blockade was attained over 60 min. In the second test, subjects underwent a euglycaemic hyperinsulinaemic clamp for 180 min, the last 60 min combined with trimethaphan (INS+TRI). Group 2 (n=8) only underwent a clamp for 180 min (INS alone). Effects of INS+TRI on haemodynamic parameters and on tritium labelled norepinephrine (NE) kinetics were compared to effects of TRI alone and INS alone. Glucose infusion rate and forearm glucose uptake during INS+TRI was compared to INS alone.

Results: Autonomic blockade with TRI expectedly and significantly decreased arterial and venous NE level, total body and limb NE spillover, and forearm vascular resistance (FVR, by 29±6%). Trimethaphan during insulin infusion (INS+TRI) decreased NE kinetic parameters as well, but to a lesser extent than during TRI alone. During INS+TRI, FVR decreased more than during TRI alone (by 59±8%, P<0.03 vs TRI) or INS alone. INS+TRI decreased diastolic blood pressure significantly more than TRI alone (10.5±2.4 vs 3.6±2.0 mmHg, P=0.02). Whole body glucose uptake was similar in both groups from 0-120 minutes, but increased significantly from 120-180 min during INS+TRI (autonomic blockade), compared to INS alone. Similar findings were obtained for forearm (skeletal) glucose uptake.

Conclusions: Sympathetic post-ganglionic blockade unmasks a potent vasodilator effect of insulin in humans and increases insulin sensitivity.

OP 32

Clinical Retinopathy

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DECREASING INCIDENCE OF DIABETES-RELATED BLINDNESS IN WORKING AGE IN THE PROVINCE OF TURIN. AGE-PERIOD-COHORT ANALYSIS OF TEMPORAL TRENDS IN 1968-1997

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Background and Aims: Reducing diabetes-related blindness (DRB) by 1/3 over 5 years was a target of the Saint-Vincent Declaration (SVD) but few data are available on the outcome of prevention programmes in Europe. This work aimed at assessing temporal trends of DRB incidence over 1968-97 in the Turin Province and establishing whether intensifying SVD-related activities in the area helped decrease DRB in 1993-97. **Methods:** Age-period-cohort analysis of data sourced from the files of the Provincial Agency granting blindness benefits. **Results:** Of 6801 incident cases of legal blindness (visual acuity ≤1/20) at age ≥35 (incidence rate 15.45/100,000 person-years, 95% CI 15.09-15.83), 785 were due to diabetic retinopathy (1.78/100,000 person-years, 1.66-1.91). DRB incidence rates increased linearly from calendar period 1968-72 to 1988-92. They declined in 1993-97 at ages 45-69 but continued to rise at ≥70. The relative risks of DRB in 1993-97 vs. 1988-92 were 0.64 (0.49-0.82) for ages 45-69 and 1.11 (0.84-1.48) at ≥70. By contrast, incidence of blindness from all causes decreased in 1993-97 in all age groups, even when cataract, glaucoma and myopia were considered separately. The best fitting model, after correction for overdispersion, included a cohort effect (polynomial, degree two) for DRB and both a period (polynomial, degree five) and cohort effects (polynomial, degree two) for all-cause blindness. **Conclusions:** This is the first report on achieving an SVD target in a European area, with a 36% drop in DRB incidence in working age. Persisting DRB above age 70 suggests that prevention and treatment should now be targeted specially at older people.

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DECREASING INCIDENCE OF DIABETIC NEPHROPATHY AND PROLIFERATIVE RETINOPATHY IN TYPE 1 DIABETES

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Background and Aims: Conflicting evidence of a so-called calendar effect on incidence of diabetic microangiopathy has been reported. To assess recent trends in the cumulative incidence of the severe microvascular complications in type 1 diabetes mellitus (DM), we analysed data from two long-term prospective observational studies. **Materials and Methods:** Six hundred patients with onset of type 1 DM between 1965 and 1984, resided in Copenhagen, were followed to 2000 or until death. Patients were divided into four cohorts based on time of DM onset as shown in the table. Group A, B, C are prevalence cohorts identified in 1984 for a clinic based follow up study. Group D is an inception cohort of all newly diagnosed type 1 DM patients referred to our clinic. **Results:** After 20 years of DM, the cumulative incidence (life-table method) of diabetic nephropathy (persistent albuminuria > 300 mg/24h) and proliferative retinopathy was reduced in patients with increasing calendar year of diabetes onset, p < 0.001 respectively.

Onset of DM	n	Cumulative incidence (95 % CI) after 20 years of DM	
		Diabetic nephropathy	Proliferative retinopathy
A: 1965-1969	113	31.1 % (22.5-39.7)	32.0 % (23.4-40.6)
B: 1970-1974	130	27.6 % (19.8-35.4)	30.3 % (22.2-38.4)
C: 1975-1979	113	18.9 % (10.9-26.9)	20.1 % (11.9-28.3)
D: 1979-1984	244	14.7 % (8.7-20.7)	10.5 % (4.9-16.1)

In the latter cohort (D vs. C vs. B vs. A), HbA1c was lower: 8.5 vs. 8.9 vs. 8.8 vs. 8.9 % (p < 0.01), time from onset of diabetes to initiation of antihypertensive treatment was shortened: 13.3 vs. 13.9 vs. 14.8 vs. 16.9 years (p < 0.001), mean BP reduced: 95.4 vs. 98.4 vs. 100.1 vs. 102.5 mm Hg (p < 0.001), and the prevalence of smokers decreased: 45 vs. 65 vs. 54 vs. 69 % (p < 0.001). **Conclusions:** During the past decades the cumulative incidence of diabetic nephropathy and proliferative retinopathy has decreased in type 1 DM. Improved glycaemic control, early aggressive antihypertensive treatment, lower BP and reduced prevalence of smoking may all contribute to the beneficial findings.

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Title: THE RELATIONSHIP BETWEEN SOLUBLE E-SELECTIN AND MICROVASCULAR COMPLICATIONS IN TYPE 1 DIABETIC PATIENTS
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Background and Aims: E-selectin is expressed on activated endothelium. Soluble E-selectin (sE-selectin), possibly cleaved from the cell surface, is raised in diabetic patients. Very few prior studies examined the relationship between sE-selectin and microvascular complications. The aim of this study is to examine whether sE-selectin is independently related to microvascular complications. **Materials and Methods:** A nested case-control study was conducted using 539 type 1 diabetic patients of the EURODIAB Prospective Complications Study (PCS). The mean age at follow-up was 40 years and the duration of diabetes 22 years. Retinopathy was assessed by centrally graded retinal photographs. Albumin excretion rates (AER) were measured centrally from 2 overnight urine collections and albuminuria was defined as an AER > 20 µg/min. Concentrations of sE-selectin were measured in duplicate by enzyme-linked immunosorbent assay kits (R&D systems). **Results:** sE-selectin was significantly correlated with WHR, diastolic blood pressure, HDL-cholesterol, creatinine, but mostly with HbA_{1c} (0.26, $p=0.0001$). Univariate models showed that increased levels of sE-selectin (> 41 ng/ml) were related to retinopathy (OR=2.1, $p=0.0005$) and albuminuria (OR=2.1, $p=0.0003$). After adjustment for HbA_{1c}, the relationships between sE-selectin and retinopathy (OR=1.4, $p=0.12$) and between sE-selectin and albuminuria (OR=1.5, $p=0.09$) were less strong and no longer statistically significant. Other confounders did not attenuate the relationship between sE-selectin and microvascular complications to the same extent. The relationship between HbA_{1c} and retinopathy or albuminuria was not affected by adjusting for sE-selectin (OR=1.8, $p=0.0001$). **Conclusions:** There is a strong relationship between sE-selectin and diabetic microvascular complications, which is no longer significant after adjustment for HbA_{1c}. The strong relationship between sE-selectin and HbA_{1c} supports the concept that sE-selectin is raised with poor glycaemic control, which may affect endothelial cell activity and subsequently microvascular complications.

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Retinopathy 10 years after diagnosis of diabetes in young adults.
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Background and Aims: The question as to whether current clinical practise reduces the incidence of retinopathy has to be clarified. The Diabetes Incidence in Sweden Study Group (DISS) follows the incidence of diabetes in young adults. We have now followed up the development of retinopathy in those diagnosed 1987-88. The aim of the study was to establish the frequency of retinopathy 10 years after diagnosis of diabetes in young adults.

Material and Methods: DISS includes all newly diagnosed 15-34 year old diabetic patients in Sweden. During 1987-1988, 806 patients were diagnosed and 582 of them (72%) agreed to be followed up 10 years later. Retinopathy data (alternative Wisconsin classification) were available in 523 patients. In 481 patients photographs taken at local hospitals were used in the assessment whereas in the remaining 42 patients only ophthalmology data were available.

Results: Ten years after diagnosis, 324 (38%) of 532 examined patients had retinopathy. The retinopathy was mild in 168 (32%), whereas 24 (5%) had moderate-severe non-proliferative retinopathy (NPDR) and 6 (1%) had proliferative retinopathy (PDR). Patients with retinopathy had a higher mean HbA_{1c}; 8.1 (1.5) % than patients without retinopathy (6.8 (1.2)%; ($p < 0.001$). Coded anonymous data were received from 57 patients who had refused to be followed up. Among non-participants 24 (42%) had retinopathy; 15 (26%) had mild, 2 moderate-severe NPDR, and 6 patients (11%) had PDR. The prevalence of retinopathy was not increased in non-participants; however, the prevalence of PDR ($p=0.002$) was highest in non-participants.

Conclusions: Despite modern treatment 40% of young adults develop retinopathy during the first 10 years of diabetes. Our observation that retinopathy was associated with high HbA_{1c} value indicates that current treatment has to be refined.

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GLYCEMIC EXPOSURE REQUIRED FOR DEVELOPMENT OF RETINOPATHY IN CHILDREN WITH TYPE 1 DIABETES
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Background and Aims: The degree of glycaemic exposure required to observe clinically significant retinal changes is unknown. This prospective study was designed to determine the lifetime cumulative glycaemic exposure (calculated as A_{1c} months) required for the development and progression and severity of retinopathy in type 1 diabetes (DM).

Materials and Methods: 115 children under the age of 15 were followed since diagnosis of type 1 DM. Severity of RP graded from fundus photographs using modified Airlie House classification (ETDRS), glycaemic control, blood pressure and UAER were assessed after duration of DM 11±1 yrs. These measurements were repeated 10 yrs later in 72 patients (M/F=40/32).

Results: At the first examination, 34 (47%) had no RP (ETDRS 10), 35% had mild (ETDRS 15-23), and 13 (18%) moderate changes (ETDRS 31-43). Of those without RP, 22 remained such or developed only mild changes (ETDRS 15-23) during the 10 yrs (Gr1). Of those without RP or with mild changes, 22 progressed slightly (ETDRS 31-43) (Gr2). The rest 38, without RP or with minimal to moderate changes (ETDRS 10-43) progressed to moderately severe or pre-/proliferative RP (ETDRS 43-65) (Gr3). The groups were identical with respect to gender, age of onset and duration of DM, BMI, blood pressure, insulin dose and single HbA_{1c} at both examinations. A_{1c} months was highest ($p<0.01$) in Gr3 (657±33) vs Gr2 (435±47) and Gr1 (376±39) at the first examination. Also the increase in A_{1c} months during the following 10 yrs was highest ($p<0.01$) in Gr3 (562±38) vs Gr2 (315±26) and Gr1 (265±32).

Conclusions: Significant progression of RP to moderately severe or pre-/proliferative RP was observed after 1219±50 (HbA_{1c} 5.1±0.2% over normal for 20 yrs), development of moderate and mild RP required 750±65 and 641±59 A_{1c} months (HbA_{1c} 3.1±0.3% and 2.7±0.2% over normal for 20 yrs, respectively).

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RESULTS OF THE FIRST-PASS OF AN ENGLISH DISTRICT WITH A DIGITAL-CAMERA-BASED RETINAL SCREENING PROGRAM.
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Background: The Bury and Rochdale Diabetic Screening Program began in April 2000. **Materials and Methods:** A Topcon TRC-NW5s non-mydratic digital camera is used. Images are assessed by a trained Staff Grade in Ophthalmology and then stored on CD-ROM. The camera is moved between a single Health Centre in each Primary Care Group area. A seven-person team led by a Consultant Ophthalmologist runs the screening service. After checking of visual acuity four mydratic digital images of each retina are taken. **Results:** Presently, 5274 diabetic patients identified from GP records have been invited for screening from a population of 303,280 (1.7%). Of these, 3700 attended (70.2%); 11.2% did not attend (DNA); 5.4% were attending ophthalmic services, were blind or refused to attend; and 13.2% were not screened for other reasons (eg. housebound). Outcomes were graded as: no diabetic retinopathy (NDR); background (BDR); pre-proliferative (PPDR); proliferative (PDR); maculopathy or ungradeable. The first wave of screening gave the following results from 3700 patients: 74.1% NDR; 20.8% BDR; 2.3% maculopathy; 0.7% PPDR; 0.8% PDR. This led to 256 patients (including ungradeable) being referred to the assessment clinic. Of these, 19 DNA'd, 40 required laser of whom 39 attended. Quality assurance measures have included internal and external review of 40 patients' photographs (1.1%). **Conclusions:** These data demonstrate that the community-based eye-screening program identifies unknown disease thereby preventing visual loss and is an effective way to improve the management of this diabetic-microvascular complication.

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Regulation of Beta-Cell Function

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DENATONIUM, THE MOST BITTER SUBSTANCE KNOWN, STIMULATES INSULIN SECRETION IN RAT PANCREATIC ISLETS AND HIT-T15 CELLS

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Background and Aims: In taste cells, sweet and bitter tasting compounds are detected by G-protein linked receptors. In these cells, denatonium, the most bitter substance known activates the G-proteins gustducin and transducin. As both gustducin and transducin are present in pancreatic beta-cells and have as yet no known function, we studied the effect of denatonium on insulin secretion.

Materials and Methods: Rat pancreatic islets, isolated by collagenase digestion, and HIT-T15 cells were studied under both static and perfusion conditions. Insulin secretion was measured by RIA. Cyclic AMP was measured by RIA. Protein was measured by Bradford assay. Cell membrane potentials and currents were measured under current clamp or voltage clamp using either whole cell or perforated patch configurations.

Results: In rat pancreatic islets, denatonium had no effect on insulin secretion under basal conditions. However, denatonium potentiated glucose-stimulated insulin release in a concentration-dependent manner up to a maximal effect at 300 μM ($n=5$; $P<0.01$). Similar results were obtained with the HIT-T15 cell. The response to denatonium was blocked by 1 μM norepinephrine ($n=4$; $P<0.01$), implying a physiological release mechanism, and by 1 μM nifedipine ($n=4$; $P<0.02$), suggesting an action via L-type voltage-dependent Ca^{2+} channels. Denatonium did not affect cyclic AMP levels. Electrophysiological studies demonstrated that denatonium depolarized the HIT-T15 cells but had no direct effect on the L-type channel currents, thus providing an explanation for the inhibitory effect of nifedipine. The depolarization was due to closure of the KATP channel and activation of a DIDS-sensitive chloride conductance.

Conclusions: Denatonium acts on the KATP channel and a chloride channel in its action to depolarize the beta-cell and stimulate insulin release. The signal transduction mechanisms underlying these novel effects remain to be elucidated.

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Overexpression of Na/Ca exchange shapes stimulus-induced cytosolic Ca^{2+} oscillations in insulin producing BRIN-BD11 cells

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Background and Aims: In response to glucose, mouse β -cells display slow oscillations of the membrane potential and $[\text{Ca}^{2+}]_i$, whilst rat β -cells display a staircase increase in these parameters. Mouse and rat islet cells differ also by their level of Na/Ca exchanger activity. The view that the inward current generated by Na/Ca exchange shapes stimulus-induced electrical activity and $[\text{Ca}^{2+}]_i$ oscillations in pancreatic β -cells was examined in insulin producing BRIN-BD11 cells overexpressing the Na/Ca exchanger.

Materials and Methods: BRIN-BD11 cells were stably transfected with NCX1.7, one of the exchanger isoform identified in the β -cell. Overexpression could be assessed at the mRNA and protein level.

Results: Appropriate targeting to the plasma membrane could be assessed by microfluorescence and the increase in Na/Ca exchange activity. In response to K^+ , overexpressing cells showed a more rapid increase in $[\text{Ca}^{2+}]_i$ on membrane depolarisation as well as a more rapid decrease of $[\text{Ca}^{2+}]_i$ on membrane repolarisation. Thus, while the rate of $[\text{Ca}^{2+}]_i$ increase averaged $10.1 \pm 0.2 \text{ nM/s}$ ($n=94$) in control cells, it averaged $17.8 \pm 0.3 \text{ nM/s}$ in NCX1.7 overexpressing cells ($n=106$, $P<0.001$). Likewise, the decrease in $[\text{Ca}^{2+}]_i$ seen on membrane repolarisation was $7.0 \pm 0.2 \text{ nM/s}$ and $5.2 \pm 0.2 \text{ nM/s}$, in overexpressing and control cells, respectively ($P<0.001$). The decrease also occurred about 30 sec earlier. In response to glucose and tolbutamide, control BRIN cells showed large amplitude $[\text{Ca}^{2+}]_i$ oscillations. In contrast, overexpressing cells showed a staircase increase in $[\text{Ca}^{2+}]_i$ without such large oscillations. Diazoxide-induced membrane hyperpolarisation restored large amplitude $[\text{Ca}^{2+}]_i$ oscillations in overexpressing cells.

Conclusions: The present data confirm that Na/Ca exchange plays a significant role in the rat β -cell $[\text{Ca}^{2+}]_i$ homeostasis, the exchanger being a versatile system allowing both Ca^{2+} entry and outflow. They show that the current generated by the exchanger shapes stimulus-induced membrane potential and $[\text{Ca}^{2+}]_i$ oscillations in insulin secreting cells, the difference in electrical activity and $[\text{Ca}^{2+}]_i$ behaviour seen in mouse and rat β -cells resulting in part from a difference in Na/Ca exchange activity between these 2 cells.

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PKA-DEPENDENT AND -INDEPENDENT STIMULATION OF EXOCYTOSIS BY GLUCAGON IN PANCREATIC B-CELLS

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Background and Aims: Exocytosis in B-cells within intact islets is both more rapid and of greater magnitude than observed in isolated B-cells. Here we explore whether this difference can be accounted for by paracrine mechanisms exerted by glucagon secreted by neighbouring A-cells.

Material and Methods: Exocytosis was measured in single B-cell as an increase in cell capacitance ($\bullet \text{Cm}$) elicited by voltage-clamp pulses from -70 mV to 0 mV .

Results: Depolarising pulses lasting 5–450 ms were applied under control conditions, in the presence of glucagons (10 nM) and the PKA-antagonist Rp-cAMPS (10 mM). In perforated patch recordings, glucagon increased $\bullet \text{Cm}$ elicited by a 100-ms pulse from $12 \pm 2 \text{ fF}$ ($n=10$) to $34 \pm 9 \text{ fF}$ ($n=5$; $P<0.01$), an effect that was not blocked by Rp-cAMPS. Glucagon had no effect during depolarisations shorter than 100 ms. A PKA-dependent component was observed during depolarisations $\bullet 200 \text{ ms}$. Similar results were obtained when exocytosis was elicited by a train of ten 500-ms depolarising pulses (1 Hz stimulation). The total increase in membrane capacitance elicited by the train fell from $154 \pm 23 \text{ fF}$ in the presence of glucagon to $52 \pm 16 \text{ fF}$ ($P<0.02$) after addition of Rp-cAMPS. However, the $\bullet \text{Cm}$ evoked by the first pulse was unaffected by the PKA-inhibitor. Stimulation of exocytosis by intracellular application of 0.1 mM cAMP during standard whole-cell recordings revealed a prominent and rapid cAMP-dependent component detectable within 30 ms ($4 \pm 1 \text{ fF}$ under control and $18 \pm 7 \text{ fF}$) that was insensitive to PKA inhibition and fully depleted within 150 ms. At longer depolarisation, a secondary PKA-dependent enhancement was observed. **Conclusions:** Our data suggest that enhanced exocytosis in intact islets in part can be attributed to the presence of glucagon. The action of glucagon involves both rapid ($<150 \text{ ms}$) PKA-independent and slow ($>150 \text{ ms}$) PKA-dependent mechanisms. However, glucagon alone is insufficient to completely restore the rapid component suggesting the contribution of additional mechanisms.

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Phosphorylation dependent intranuclear shuttling of PDX1 in pancreatic beta cells.

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Background and Aims: Insulin gene transcription is regulated partly through activation of the homeodomain transcription factor PDX1 by way of a signalling pathway involving both PtdIns 3-kinase and SAPK2/p38. Previous studies have shown that glucose, insulin and the cellular stress agent sodium arsenite can activate PDX1, promoting DNA binding. Following activation, PDX1 transfers from an inactive cytoplasmic form to an active form, permitting its translocation to the nucleus and stimulation of insulin gene transcription. The present study was undertaken to investigate the intranuclear trafficking of PDX1 in a human beta cell overexpressing PDX1 and in MIN6 cells.

Results: Immunocytochemistry was used to localise PDX1 to the nuclear periphery in low glucose. Following stimulation with high glucose, PDX1 was present in the nucleoplasm. Nuclear translocation of PDX1 was time and dose responsive occurring within 10 minutes and 5mM glucose. Insulin and sodium arsenite also stimulate movement of PDX1 from the nuclear periphery to the nucleoplasm. In cells transferred between high and low glucose concentrations, PDX1 was found to shuttle rapidly between the nuclear periphery and the nucleoplasm. The effect of glucose and insulin on the nuclear localisation of PDX1 was inhibited by wortmannin and SB203580, confirming that a pathway involving PtdIns 3-kinase and SAPK2 was involved, but was unaffected by PD098059 or rapamycin, inhibitors of the MAPK pathway and p70s6k respectively. Movement from the nucleoplasm to the nuclear periphery was inhibited by calyculin A and okadaic acid suggesting dephosphorylation dependent translocation of PDX1 was involved.

Conclusions: These results demonstrate that PDX1 shuttles between the nuclear periphery and the nucleoplasm in response to changes in glucose and insulin concentrations and that these events are dependent on PtdIns 3-kinase and SAPK2.

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Wortmannin sensitive gene regulation by the transcription factor PDX-1 mediates glucose dependent activation of the rat insulin-1 promoter by glucagon-like peptide 1

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Background and Aims: Glucagon-like peptide 1 (GLP-1) acts as an insulinotropic hormone in a glucose dependent manner to maintain insulin biosynthesis, gene expression, and secretion in pancreatic beta-cells. This has been proposed to be mediated through a cAMP dependent signalling pathway. In previous studies, however, we have obtained evidence that glucose dependent transactivation of the rat insulin 1 promoter by GLP-1 is not cAMP dependent. Here we report that this effect of GLP-1 is mediated by the homeodomain transcription factor PDX-1 through a wortmannin sensitive signalling pathway. **Materials and Methods:** Glucose dependent signal transduction by GLP-1 was analysed in INS-1 beta-cells and human pancreatic islets by reverse transcription polymerase chain reaction (RT-PCR), luciferase reporter gene assays, gel shift assay, DNase I footprint assay and fluorescence microscopy using PDX-1-green fluorescent (GFP) fusion proteins. **Results:** In human islets we find induction of proinsulin mRNA by GLP-1 (10 nM) at 11.1 mM but not at 2.5 mM glucose, and this induction can be inhibited by the PI3 kinase inhibitor wortmannin (10 nM). Glucose dependent transactivation of the rat insulin 1 promoter by GLP-1 is also inhibited by wortmannin in INS-1 beta-cells. In contrast, induction of proinsulin mRNA and promoter activity by the cAMP mimetic forskolin (10 µM) was not glucose-dependent. Further, we demonstrate that the glucose dependent gene regulatory effect of GLP-1 is dependent on the presence of PDX-1 binding sites (A-elements) within the promoter. Results from fluorescence immunocytochemistry, electrophoretic mobility shift assays (EMSA) and DNase I footprint analysis in INS-1 beta-cells further indicate, that wortmannin inhibits GLP-1 mediated nuclear translocation and DNA-binding of PDX-1 in a glucose dependent manner. **Conclusions:** We provide evidence that GLP-1 dependent gene regulation in pancreatic beta-cells occurs through both a cAMP dependent, glucose independent, pathway that maintains basal insulin gene expression, and a wortmannin sensitive PI3-kinase dependent signalling pathway, that involves the transcription factor PDX-1 and mediates glucose dependent proinsulin gene expression and may also mediate beta-cytotropic effects of GLP-1 in the endocrine pancreas. These results contribute to the understanding of GLP-1 effects at pancreatic beta-cells, because GLP-1 analogues are currently being developed as treatment for diabetes mellitus.

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Proinsulin biosynthesis is regulated at the level of mRNA translation and stability involving an RNA-protein interaction

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Background and Aims: Proinsulin biosynthesis is regulated in response to glucose, in the short term (< 2 hours) through changes in the translation of the preproinsulin (ppl) mRNA and in the longer term (> 6 hours) through changes in ppl mRNA levels. Recently, we showed that the ppl mRNA untranslated regions (UTRs) are necessary for this translational regulation. The 5'UTR promotes translation as glucose levels rise, while sequences within the 3'UTR, that suppress translation at low glucose, are overcome. Sequences within the 3'UTR were also shown to influence ppl mRNA stability. In the present study we extended our analysis of proinsulin biosynthesis by examining the role of ppl mRNA sequences that regulate the translation and stability of the mRNA.

Materials and Methods: Translation rates were determined by [³⁵S] methionine labelling, immunoprecipitation, gel electrophoresis and quantification by phosphorimaging. RNA levels were measured by RNase protection assay. RNA gel-shifts were performed using [³²P]UTP labelled RNAs incubated with protein extracts with RNA-protein complexes being resolved by native gel electrophoresis.

Results: A recombinant adenovirus expressed an mRNA, in the β-cells of isolated rat islets, in which the ppl mRNA UTRs flanked the luciferase coding region. Islets infected with this adenovirus, cultured at 2.8 or 11.1 mM glucose showed that the ppl mRNA UTRs alone could confer glucose regulation of luciferase translation. Analysis of the ppl mRNA levels revealed that the rat preproinsulin gene promoters are resistant to actinomycin D inhibition. Expression of an adenovirus encoding CMV promoter driven his-tagged preproinsulin encoding mRNA allowed separation of endogenous and tagged preproinsulin mRNAs. Actinomycin D inhibition of the CMV promoter showed that the stability of the tagged preproinsulin mRNA was regulated in response to glucose. Since these and previous studies have implicated the 3'UTR in the regulation of ppl mRNA translation and stability RNA gel-shift analysis examined 3'UTR sequences for RNA-protein interactions. A protein/3'UTR interaction was detected and shown by competition and mutational analysis to be with a sequence conserved between mammalian ppl mRNAs (UUGAANNAGC). This RNA-protein interaction was shown to be glucose regulated. **Conclusions:** These data show that proinsulin biosynthesis is regulated at the level of translation through elements in the ppl UTRs and by the stability of the ppl mRNA. These processes are likely to be regulated by protein interactions, such as that which we have shown occurs in a glucose dependent manner with a conserved cis-element within the 3'UTR.

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Genetics of Type 1 Diabetes

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Development of an in vitro model to investigate the structure/function relationship of the HLA-DQ6.2 molecule

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Background and Aims: The HLA-DQ6.2 molecule (DQA1*0102/DQB1*0602) confers natural protection against type 1 diabetes, but the HLA-DQ6.4 molecule (DQA1*0102/DQB1*0604) does not. These molecules are structurally similar, differing at only six amino acid residues in the beta peptide chain. Computer modelling studies have suggested that the disparate disease associations of DQ6.2 and DQ6.4 may be attributed to amino acid differences at residues beta57 and beta70. We have devised an in vitro model of the DQ6.2 molecule which will allow us to determine how these two residues affect the function of the molecule in the immune response and how this may influence its effect on disease risk. **Materials and Methods:** The DQA1*0102 and DQB1*0602 alleles were cloned into the pCIneo expression vector. The DQB1*0602 allele was subjected to site-directed mutagenesis at codons 57 and 70 to create two mutant alleles. Each mutant allele encodes a peptide chain in which a single amino acid residue has been substituted with the corresponding residue from DQ6.4. The appropriate DQB1 allele (wild-type or mutant) was paired with the DQA1 allele and introduced into the HLA class II-negative B cell line, BLS-1, by electroporation. Stably transfected cells expressing DQ were isolated using magnetic beads and cloned by limiting dilution. **Results:** Three batches of transfected cells were successfully produced using this technique; a) those expressing wild-type DQ6.2, b) those expressing mutant DQ6.2 with Val at residue beta57 and c) those expressing mutant DQ6.2 with Arg at residue beta70. **Conclusions:** The methodology described above can be used to express DQ6.2 in isolation from other HLA class II molecules and permits the genetic manipulation of its structure. The transfectants created will be used to investigate the effect of each amino acid substitution on the function of the DQ molecule. The findings will help to determine how the structure of DQ6.2 is important for its role in conferring protection against type 1 diabetes.

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FINE MAPPING OF THE NON-CLASS II HLA GENE ASSOCIATED WITH TYPE 1 DIABETES MELLITUS

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Background and Aims: Existence of non-DQ-DR genes associated with type 1 diabetes mellitus (T1DM) in HLA is now established. Recently we were able to localize a putative non-DQ-DR gene in the 240 kb of to the centromeric end of the HLA class I region. Candidate genes, HLA-B, HLA-C, MICA and MICB are located within this region. Our goal is to study all SNPs in these highly polymorphic genes in order to find which one is associated with T1DM.

Materials and Methods: 75 patients and 181 controls were stratified for the DR3/4(0404) genotype, and 241 patients and 354 controls stratified for the DR3/4(0401) genotype (all from Finland). These were matched in order to exclude the effect of strongly T1DM-associated DQ-DR genes. Genotyping is done by means of sequencing followed by sequence reads alignment using Staden package and manual base-call checking at the potential polymorphic sites.

Results: So far SNPs of the MICA gene were studied in the DR3/4(0404)-stratified group of patients and controls. We were able to exclude 30 SNPs of 56 known to be present in exons 2, 3 and 4 as well as introns 2 and 3 of the MICA gene. Twenty of these SNPs were not polymorphic in the studied cohort, while 10 had rare minor alleles, which were not in linkage disequilibrium with the previously identified susceptible haplotype, marked by DRB1*0404 - B*39 alleles.

Conclusions: Future study of the DR3/4(0401)-stratified group of patients and controls will allow comparison of the allele effect of the other 26 SNPs on DR4(0404) and DR4(0401) haplotypes and eventually exclude most of the SNPs in MICA gene as well as other candidate genes in the 240 kb HLA region.

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A novel vitamin D receptor gene polymorphism confers susceptibility to type 1 diabetes mellitus.

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Background and Aims: Autoimmune destruction of pancreatic beta cells causes type 1 diabetes. Administering vitamin D prevents the onset of autoimmune diabetes in NOD mice. 1,25(OH)₂D₃ decreases HLA class II expression on pancreatic beta cells, inhibits cytokine-mediated lymphocyte proliferation and subsequent islet invasion. Vitamin D's immunomodulatory effects are mediated via its nuclear receptor (VDR). Known polymorphisms of the VDR gene were found to be associated with type 1 diabetes in Caucasians and Asians. However, alleles conferring an increased risk for type 1 diabetes vary between Asians and Caucasians suggesting the known polymorphisms to be in a differential linkage with another potentially functional polymorphism. Recently, a novel Tru9I VDR polymorphism was discovered in intron 8. The aim of this study was to further elucidate the role of genetic VDR variants in type 1 diabetes in general and of the novel Tru9I polymorphism in particular.
Materials and Methods: 285 Caucasian pedigrees with an affected offspring were genotyped for two established (BsmI, FokI) polymorphisms and the novel Tru9I site using a PCR-RFLP approach. Allele and genotype frequencies were determined and pairwise linkage disequilibria (LD) were calculated. Indirect haplotyping and (extended) transmission disequilibrium testing ((E)TDT) was performed. **Results:** Observed allele frequencies were in accordance to previous reports (79.2% TT, 19.7% Tt, 1.1% tt) and linkage disequilibria between the polymorphisms were low (LD=0.005-0.039). Allele 'u' was significantly less often transmitted to affected offspring than expected (36 times transmitted vs. 59 times not transmitted; TDT: p=0.018). The overall transmission pattern of extended BsmI/Tru9I haplotypes differed significantly from expected values (ETDT: p=0.025). In general, alleles containing 'u' were less often transmitted to diabetic offspring than expected ('bu': 25 transmitted vs. 42 not transmitted, TDT: p=0.038; 'bfu': 10 transmitted vs. 21 not transmitted, TDT: p=0.048), but BsmI and -FokI appear to contribute to this association only to a lesser extent. **Conclusions:** This study suggests the novel Tru9I VDR polymorphism to confer susceptibility to type 1 diabetes in Caucasians. Linkage analyses with additional VDR polymorphisms and functional studies are required to elucidate the role of this novel VDR variant and the potential of vitamin D analogues in preventing type 1 diabetes mellitus.

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GENE EXPRESSION STUDIED BY cDNA MICROARRAY IN INSULIN-SENSITIVE TISSUES IN STREPTOZOTOCIN-INDUCED DIABETES

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Background and Aims: Complete understanding of the mechanism of insulin action on cellular level is fundamental for understanding the etiology of insulin resistance in diabetes. For instance, the regulation of the mechanism of insulin-mediated glucose metabolism is not yet fully known. Therefore the aim of this study was to search for novel regulatory genes involved in the insulin-regulated glucose metabolism. The search was based on cDNA microarray.

Materials and Methods: Male Sprague-Dawley rats were injected with streptozotocin i.p. (80 mg/kg) to induce type 1 diabetes. Seven days later the samples from control and diabetic skeletal muscle, cardiac and adipose tissues as well as liver were taken and used as RNA source. Derived cDNAs were hybridized to a rat gene filter (Research Genetics) containing both known genes and EST sequences, altogether 5184 transcripts.

Results: Applying an expression difference factor 2, we found 934 down-regulated genes in insulin-deficient skeletal muscle and only one down-regulated gene in adipose tissue. Numbers of up-regulated genes were 9 and 6, respectively. Skeletal and cardiac muscle shared 5 down-regulated EST sequences and 3 known genes, which were lutropin-choriogonadotropic hormone receptor, sciatic nerve integrin beta subunit and a calcium-binding protein, the last being down-regulated in liver, too. 4 EST sequences and 2 known genes (carboxypeptidase-a and ras-related GTPase Rab29) were down-regulated in diabetic skeletal muscle and liver. Munc 18-1 gene was down-regulated in cardiac muscle and liver. No mutual up-regulated genes were found in different tissues.

Conclusions: These preliminary results that need to be confirmed by independent methods show the expected down-regulation of various genes due to insulin-deficiency. Further, the results suggest that cDNA-array analysis is a potential method for identifying genes involved in the metabolic disturbances in diabetes.

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LINKAGE DISEQUILIBRIUM OF AN INTERLEUKIN-12 POLYMORPHISM (IDDM 18) IN DANISH TYPE 1 DIABETES FAMILIES.

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Background and Aims. Interleukin 12 (IL-12) drives the differentiation of T-lymphocytes into the Th1-subset, characterized by production of cytokines leading to cell mediated immunity. In the NOD-mouse IL-12 has been shown to play a primary role in Type 1 diabetes (T1D) induction. Lately, based on the results from the NOD-mouse, a new human T1D susceptibility locus – IDDM 18 – has been identified, positioned near the IL-12 P40 gene (*IL12B*) on chromosome 5q33-34. Linkage to human T1D has been shown in the region and a polymorphic TaqI site in the 3'UTR of this gene shows preferential transmission to T1D offspring, examined in an Australian and British family material. The polymorphism is biallelic. Increased IL-12 production has been shown for 1/1 homozygous compared to 2/2 homozygous people, in EBV transformed cell lines. The aim of our study was to test this polymorphism in a homogeneous Danish family material, in order to see if we can confirm the finding.

Material and Methods. We typed 254 Danish T1D families, comprising 102 simplex families and 152 sib-pair families. PCR-amplification was used, followed by TaqI digestion at 65° for 12 hours and analysis on 2% agarose gel. Data was analysed by transmission disequilibrium test (TDT) and Sib-TDT tests, addressing an eventual preferential transmission of one allele vs. another.

Results. TDT revealed preferential transmission of allele 1 (99 vs. 70 transmissions of allele 2) to affected children. (Chi-square 4.98, p=0.026). Combining with the sib-TDT gave a Z' score of 2.35, p=0.019.

Conclusion. The allele 1 of the IL-12 polymorphism in a Danish family material, confirmed preferential transmission to affected children, suggestive of linkage to T1D. Whether this variant itself represents the IDDM 18 susceptibility locus, or whether it is in linkage disequilibrium with the real T1D causing variant is not known. As this polymorphism is considered an important putative marker for T1D, it is important to confirm the involvement of the *IL12B* polymorphism in T1D in several populations. Additional functional studies are needed to define possible pathogenetic implications and potential future intervention strategies.

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Common genes in type 1 and type 2 diabetes: Lessons from cross hybrids between diabetic BB and WOKW rats developing a metabolic-like syndrome.

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Background and Aims: It is well-known that type 1 and type 2 diabetes including facets of metabolic syndrome cluster in same families. This fact may be a hint for common genes. That prompted us to cross lymphopenic (Idm2) BB/OK (RT1u=Idm1) developing an type 1-like diabetes with non-lymphopenic WOKW rats (RT1u=Idm1) developing a polygenic, complete metabolic-like syndrome.

Materials and Methods: (BB x WOKW)F1 hybrids were backcrossed onto diabetic BB/OK males resulting 152 first backcross hybrids (BC1) studied for diabetes occurrence up to an age of 30 weeks. To find out whether diabetogenic non-MHC genes mapped previously on chromosomes 18 (Idm3), 6 (Idm4) and 1 (Idm5r) are diabetogenic genes of WOKW, 71 Idm2 homozygous hybrids were genetically analysed with 29 microsatellites located on these chromosomes.

Results: 56 out of 152 hybrids (37%) developed diabetes which were lymphopenic (Idm2/Idm2). Compared with the expected diabetes frequency of 25%, significantly more BC1 hybrids developed diabetes (25% vs. 37%, p<0.001). Around Idm4 on chromosome 6 a significant difference in the allele distribution was found. More homozygotes than heterozygotes were diabetic (p=0.015). No significant difference was detected on chromosomes 18 and 1. However, hybrids heterozygous at Idm5r on chromosome 1 developed significantly later diabetes than homozygotes (95±27 vs. 81±19 d; p<0.3).

Conclusions: These findings 1) confirm Idm4 on chromosome 6, 2) suggest that WOKW gene on chromosome 1 (Idm5r) does not protect but delay diabetes development, 3) support our hypothesis of common genes in type 1 and type 2 diabetes.

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Type 2 Diabetes Genetics: Animal Models

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New mouse models of Type II diabetes generated by random N-ethyl-N-nitrosourea mutagenesis.

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Background and Aims: F1 offspring of mutagenised males from the SB/Harwell mutagenesis program and a sensitised screen utilising insulin resistant knockout mouse strains (IR, IRS-1) were assayed for impaired glucose tolerance or diabetic phenotypes. Mice with a phenotype of interest were backcrossed for inheritance testing. **Materials and Methods:** F1 mice were assayed using either free fed glucose concentrations or glucose tolerance tests (IPGTT). Mice were fasted overnight, a blood sample taken and a glucose load of 2g/Kg administered via IP injection; subsequent blood samples were taken over a 2 hour time course. DNA was extracted from tail biopsies and used for genetic mapping studies. For selected mutants, mice at 15 months of age were fasted, injected with insulin under a terminal anaesthetic and tissues removed for protein signalling assays and histology. Pancreata from these mice were fixed and serial sections obtained, H&E stained, and in-situ hybridisation of glucagon and insulin riboprobes carried out. **Results:** Fifteen male mice were identified from the Harwell mutagenesis program on the basis of high free fed glucose and crossed for inheritance testing. Two of these lines to date have been confirmed to segregate an impaired glucose tolerance phenotype and are currently being mapped. Analysis of Pancreata from one line indicates abnormal distribution of glucagon cells within the Islet. To date six mice from the sensitised screen have been identified with impaired glucose tolerance using an IPGTT. These mice are currently undergoing inheritance testing. **Conclusions:** At least two of the lines tested to date, GENA263 and GENA348 exhibit an autosomal dominant, sexually dimorphic, impaired glucose tolerance, suggesting that a random mutagenesis approach is successful in generating new Type II diabetes mouse models. Male mice in both lines exhibit impaired glucose tolerance, with GENA263 mice progressing into a type II diabetic phenotype with increased age. Female mice with normal body weight have normal glucose tolerance, only exhibiting impaired glucose tolerance with increase body weight. We hypothesise that the impaired glucose tolerance observed in these mice is primarily caused by insulin resistance, therefore, further studies are underway to identify the extent and location of insulin resistance in these mice.

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DIFFERENTIAL GENE EXPRESSION IN THE LIVER OF DIABETIC PSAMMOMYS OBESUS

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Background and Aims: *Psammomys obesus* (Israeli sand rat) is a polygenic model of obesity and type 2 diabetes. Increased hepatic glucose production contributes to the hyperglycemia in diabetic animals, suggesting a central role for the liver in the development of type 2 diabetes in these animals. We hypothesized that diabetic animals had disturbances in whole body fat and carbohydrate oxidation, and that these disturbances could be secondary to altered gene expression in the liver of diabetic animals. This study examined energy expenditure and substrate utilisation in *P. obesus* and identified genes differentially expressed in the liver of diabetic animals. **Materials and Methods:** 24-hr indirect calorimetry was used to determine energy expenditure and substrate utilisation in lean (n=10), obese (hyperinsulinemic, n=10), and obese/diabetic (n=10) *P. obesus*. Representational difference analysis (RDA) was used to identify genes differentially expressed between lean and obese/diabetic *P. obesus* (n=3 in each group). Differentially expressed genes were subsequently cloned and isolated. **Results:** Total energy expenditure was significantly elevated (p<0.05) in obese (139±22 kJ/day) and obese /diabetic (133±5 kJ/day) animals relative to lean controls (104±6 kJ/day). Fat oxidation was elevated in obese (0.7±0.1 mg/min) and obese /diabetic (0.8±0.1 mg/min) animals relative to lean controls (0.4±0.1 mg/min), reaching statistical significance (p<0.05) in obese/diabetic animals only. After RDA, 96 clones were selected, of which 6 were identified as up regulated and 5 were down regulated in livers obese/diabetic animals relative to lean controls. The relationship between these genes and energy metabolism in *P. obesus* is currently being investigated. **Conclusions:** Energy expenditure and fat oxidation were significantly increased in obese/diabetic animals relative to lean controls. 11 differentially expressed genes were identified in the liver of diabetic animals that may contribute to disturbances in energy metabolism.

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HYPOTHALAMIC BEACON GENE EXPRESSION AND THE DEVELOPMENT OF OBESITY AND TYPE 2 DIABETES

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Background and Aims: Beacon is a recently discovered hypothalamic peptide involved in the regulation of energy balance. The beacon gene is overexpressed in the hypothalamus of obese animals in proportion to body fat content, and ICV administration of beacon resulted in a dose-dependent increase in food intake and body weight. To further elucidate the physiological mechanisms of beacon action, we measured hypothalamic beacon gene expression at various developmental stages in genetically selected diabetes-resistant and diabetes-prone *Psammomys obesus* fed varying diets. **Materials and Methods:** Hypothalamic beacon gene expression was measured using TaqmanTM fluorogenic PCR in 4-, 8- and 16-week-old animals from each genetically selected line. **Results:** Beacon gene expression was elevated in the diabetes-prone compared with diabetes-resistant *Psammomys obesus* at 4 weeks of age (p=0.018) despite no difference in body weight between the groups. At 8 weeks of age, hypothalamic beacon gene expression was elevated in diabetes-prone animals fed a high energy diet (p=0.004), and was correlated with serum insulin concentration (r²=0.65, p<0.001). **Conclusions:** *Psammomys obesus* with a genetic predisposition for the development of obesity and type 2 diabetes have elevated hypothalamic beacon gene expression at an early age, suggesting that overexpression of beacon may contribute to the development of obesity and insulin resistance in these animals. We are currently conducting a range of studies to further investigate the role of beacon in the regulation of energy balance.

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Sex-specific and sex-independent quantitative trait loci (QTLs) for facets of the metabolic syndrome in WOKW rats.

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Background and Aims: WOKW rats develop a complete metabolic syndrome closely resembling human disease. Since genetic studies using male (WOKW x DA)F2 progeny showed that several independent genetic factors were involved a polygenic basis for the syndrome in WOKW was assumed. However, because the metabolic syndrome in human clearly demonstrates sex differences we have extended our study to include both male and female (WOKW x DA)F2 progeny.

Materials and Methods: 140 female F2 hybrids were phenotypically characterised for body weight, BMI, adiposity index, serum lipids, insulin, leptin and glucose tolerance at an age of 28, 30 and 32 weeks. 126 microsatellite markers were used in a genome-wide scan of F2 hybrids. Genetic linkage maps and the location of QTLs were determined with the MAPMAKER computer package.

Results: Comparing findings of these 140 females with those of 150 male F2 hybrids studied before male- or female-specific QTLs were mapped for body weight, BMI, adiposity index and serum insulin on chromosomes 1 and 5, serum triglycerides on chromosomes 4, 7, 11 and 16, serum total and HDL-cholesterol on chromosomes 3, 4, 5, 10, and 17, serum leptin on chromosomes 8 and 16 as well as blood glucose and glucose tolerance (AUC) on chromosomes 3, 4 and 17. QTLs for both, males and females were only found for body weight on chromosome 1 and for serum total cholesterol on chromosome 3 and 10.

Conclusions: These findings clearly demonstrate that there are sex-specific and sex-independent QTLs for facets of the metabolic syndrome in WOKW rats.

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Dissection of genes for type 2 diabetes using consomic strains carrying diabetogenic chromosomes 11 and 14 from diabetic NSY mice

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Background and Aims: The dissection of susceptibility genes for common multifactorial diseases, such as type 2 diabetes, is a formidable challenge. Using an inbred animal model for type 2 diabetes, the NSY mouse, we previously mapped two major QTLs (Nidd1n and Nidd2n) for glucose intolerance in large intervals of chromosomes 11 and 14, respectively. The aim of this study was to clarify the impact of these QTLs on diabetes-related phenotypes and to dissect a complex trait into genetic components.

Materials and Methods: Using a marker-assisted "speed" congenic strategy, we established two consomic strains, C3H.NSY-*chr11* and C3H.NSY-*chr14*, in which whole diabetogenic chromosomes (11 and 14, respectively) from the NSY mouse were introgressed onto the control genetic background (C3H). Diabetes-related phenotypes of the two consomic strains were compared with the control C3H strain at 48 weeks of age.

Results: C3H.NSY-*chr11* mice exhibited significantly higher blood glucose levels on fasting and after glucose challenge, significantly higher fasting insulin levels and lower insulin response to glucose than C3H mice. C3H.NSY-*chr14* mice showed significantly higher fasting insulin level than C3H mice, but their glucose tolerance was normal. Body weight and weight of epididymal fat pad of the two consomic strains were identical to those in control C3H mice, which were significantly smaller than those in NSY mice.

Conclusions: Diabetogenic gene(s) on the NSY chromosome 11 impairs glucose tolerance through both insulin resistance and insulin secretion, whereas that on chromosome 14 causes insulin resistance, but not glucose intolerance. Both QTLs cause insulin resistance independent of obesity. These data indicate that the power and importance of the consomic/congenic strategy in dissecting a complex trait into each genetic component.

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EMBRYOS OF DIABETIC PARENTS ARE DESTINED TO DEVELOP DIABETES EVEN IF TRANSFERRED INTO THE UTERUS OF A NON-DIABETIC MOTHER.

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Background and Aims: Genetic and environmental factors, including the intrauterine milieu, play a role in determining susceptibility to Type 2 diabetes. The aim of the current study was to assess the relative contributions of genes and intrauterine environment by embryo transfer technology.

Materials and Methods: Female (diabetic) Goto Kakizaki (GK) rats were superovulated by the intraperitoneal injection of 5IU of pregnant mare's serum followed 48-hours later by 5IU of human chorionic gonadotrophin. They were then mated with male (diabetic) GK rats and, the following morning, fertilized eggs were harvested from the oviducts. Subsequently embryos were microsurgically transferred into anaesthetised Wistar (non-diabetic) females that had been rendered pseudopregnant by mating with vasectomised Wistar male rats. Pregnancies were allowed to go to term and the offspring studied after weaning.

Results: A total of 47 offspring have been produced to date (litter size 3-8). Young offspring (n=46) had a mean plasma glucose of 7.1 mM (SEM 1.1) and adults (n=24) had a mean plasma glucose of 7.6 mM (SEM 1.6). This compares with mean glucose values of 7.1mM (SEM 0.2) and 8.8 mM (SEM 0.3) in young and adult GK offspring born from their natural mothers (p>0.05). GK rats born to their natural mothers and by embryo transfer had significantly higher plasma glucose levels than normal Wistar control rats (at 6-weeks, 3.5 mM (SEM 0.1) and at 4-months 4.6 mM (SEM 0.2), p<0.05).

Conclusions: There were no significant differences in the glucose levels of young or adult rats whether or not they had developed in a diabetic uterus. We conclude that in the GK rat, genetic factors outweigh the role of a normoglycaemic intrauterine environment in determining the blood glucose levels of offspring. The findings of this study imply that tight glycaemic control in gestational diabetes would not reduce the risk of diabetes in the offspring of parents who have transmitted powerful genetic determinants of the disease. Complementary experiments, in which the embryos of Wistar rats are reared in a GK uterus, are underway.

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Assessment of Insulin Secretion in Man

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POPULATION APPROACH TO ESTIMATE INSULIN SENSITIVITY AND GLUCOSE EFFECTIVENESS BY MINIMAL MODEL

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Background and Aims: Individual analysis (IND) by minimal model of glucose kinetics fails in 5-20% cases. We have employed population hierarchical Bayesian modelling (POP) with the aim to avoid these failures.

Materials and Methods: Data were analysed from 65 subjects with Type 2 diabetes (male/female 53/12, age 54±1 years, BMI 30.5±0.7kg/m²; mean±SE) who underwent frequently-sampled insulin-modified IVGTT (30 samples over 180min; 0.3g/kg glucose, 0.05mU/kg insulin at 20 min). POP facilitates simultaneous estimation of individual and population parameters. WinBUGS program carried out probabilistic Bayesian inference using vague prior distributions. **Results:** POP gave estimates of insulin sensitivity S_i in all subjects. IND failed in the 3 cases where POP-derived S_i was the lowest and in one further case located in the lower quartile. POP and IND gave comparable and highly correlated estimates of S_i ($r_s=0.98$, $P<0.001$, $N=61$) and glucose effectiveness S_g ($r_s=0.77$, $P<0.001$, $N=65$).

	N	mean (95% CI)	lower quartile	upper quartile	interquartile range
*POP- S_i	65	1.07 (0.82-1.36)	0.57	2.01	1.43
IND- S_i	61	1.23 (0.97-1.56)	0.67	2.50	1.84
**POP- S_g	65	1.53 (1.41-1.65)	1.31	1.77	0.46
IND- S_g	65	1.45 (1.31-1.58)	1.18	1.71	0.53

* S_i in $10^{-5} \text{ min}^{-1} \text{ per pmol L}^{-1}$; ** S_g in 10^{-2} min^{-1}

Interquartile range given by POP was tighter by ~20% for S_i and by ~15% for S_g . **Conclusions:** Individual analysis of insulin-modified IVGTT data fails in subjects with low to very low S_i . Population analysis is the method of choice as it avoids these failures and gives a smaller unbiased estimate of the population variance for both S_i and S_g .

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THREE INSULIN RESISTANCE INDEXES: COMPARISON AND THEIR PREDICTIVITY FOR THE PARAMETERS OF METABOLIC SYNDROME

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Background and aims: Recently, a new Insulin Sensitivity Index (ISI) based on glucose and insulin (ins) after a glucose load has been proposed (Matsuda & De Fronzo, 1999). We compared basal insulin (Ins), the HOMA index and ISI in the prediction of parameters of Metabolic Syndrome. **Patients and methods:** a consecutive series of 649 (549 F, 100 M) obese patients (BMI>30 kg/m²) aged>18 yrs, with no history of diabetes, not treated with drugs interfering with IS. Fasting and post-load glucose and ins, triglyceride (TRG), HDL cholesterol (HDLc) and blood pressure were measured. Prevalence of hypertension, hypertriglyceridaemia (hTRG), and low HDLc in the upper quartile of ISI, HOMA, and Ins was compared to that of the lowest quartile. **Results:** F had an age of 46.0±13.6 yrs; and M of 45.1±15.4 yrs, a BMI of 34.7 [31.0-40.9] kg/m² in F, and 36.1 [31.4-41.4] kg/m² in M, a WHR of 0.86 [0.82-0.90] in F, and 0.92 [0.89-0.99] in M; the prevalence of diabetes (DM) was of 18.6% in F, and 12.0% in M, of IGT was 24% both in F and M, of hypertension (treated or not) was of 44.4% in F, and 56% in M, of hTRG was of 39.9% in F, and 52% in M, of low levels of HDLc of 24.4% in F, and 17.0% in M. Glucose 0 and 120' were correlated with HOMA and ISI (p<0.01), and weakly with Ins in F, and M (p<0.05); TRG were significantly correlated with the indexes studied; HDLc was significantly correlated with Ins in F, and M, while the correlation with HOMA and ISI was statistically significant only in F. Hypertension did not show significant correlations with the indexes. The RR (upper quartile vs. lowest quartile) for DM and IGT was 2.1, 3.8, and 4.9 in F, while in M was of 2.4, 4.3, and 4.9; RR for hTRG of 1.6, 2.3, and 2.2 in F versus 5.4, 6.8, and 5.4 in M; the RR for low HDLc was of 2.8, 2.7, and 2.0 in F, and 3.4, 2.7, and 3.4 in M; none of the three indexes was significantly predictive of hypertension. **Conclusions:** Ins and HOMA resulted to be similarly predictive for hTRG and low HDLc, while HOMA was more predictive of DM and IGT. ISI, which is highly correlated with HOMA, was not more predictive for the abnormalities of the metabolic syndrome when compared to the simpler and less expensive HOMA insulin resistance index.

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Homeostasis model assessment (HOMA) as a clinical index for insulin resistance in patients with type 2 diabetes in comparison to the euglycaemic clamp

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Insulin resistance plays an important role in type 2 diabetes. The gold standard for the in vivo measurement of insulin resistance is the euglycemic hyperinsulinemic clamp technique. However, this technique is time-consuming, expensive and not suitable for epidemiologic studies. The Homeostasis model assessment (HOMA) was developed as a simple and inexpensive alternative to the more complex clamp technique. With the HOMA insulin resistance is calculated by the equation: degree of insulin resistance = fasting insulin (pmol/l) x fasting plasma glucose (mmol/l) / 22.5.

Our aim was, therefore, to evaluate, whether the determination of insulin resistance by HOMA can be used as an estimate of the degree of insulin resistance characterized by the euglycemic hyperinsulinemic clamp. We investigated 113 individuals with euglycemic hyperinsulinemic clamp, 68 patients with impaired glucose tolerance and insulin resistance and 45 insulin sensitive controls. In addition, body mass index (BMI), fasting plasma glucose, fasting insulin and C-peptide levels, and the plasma concentrations of free fatty acids (FFA), total-, HDL-, LDL-cholesterol and HbA1c levels were determined.

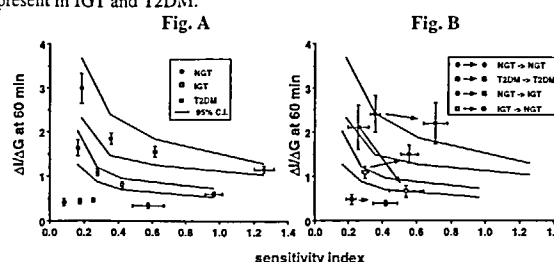
For our insulin assay (MEIA, ABBOTT) we calculated a HOMA of > 20 as insulin resistant (optimized by receiver operating characteristics (ROC)). The specificity of the model is 89.9% and the sensitivity 84.4% for our patients. The mean HOMA value was 80.17 ± 56 for individuals with insulin resistance and 14.82 ± 9.26 for insulin sensitive probands ($p=0.01$), respectively, as determined by the clamp technique. In conclusion, our data therefore suggest, that the HOMA is a suitable and simple method to reliably assess the degree of insulin resistance in epidemiological studies.

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EVALUATION OF INSULIN SENSITIVITY AND INSULIN RELEASE FROM ORAL GLUCOSE TOLERANCE TEST: BASAL STUDIES AND FOLLOW-UP OF IMPAIRED GLUCOSE TOLERANCE

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In order to derive from OGTT (75 g glucose with samplings at 0-60-120 min) indexes of both insulin sensitivity (IS) and early insulin release (IR) in normal/impaired glucose tolerance (NGT, IGT) and type 2 diabetes (T2DM), OGTTs of 337 NGT, 180 IGT, and 78 T2DM subjects were analyzed. The IS index used was: $1/\text{HOMA}$ [$405/\text{fasting insulin } (\mu\text{U/ml}) \cdot \text{glucose } (\text{mg/dl})$]; the IR index was: $\Delta\text{insulin}/\Delta\text{glucose}$ at 60 min. Values of IS were divided in quartiles for NGT, IGT, and T2DM subjects; for each quartile, early IR was calculated. Hyperbolic curves thus obtained were significantly different in NGT, IGT, and T2DM (Fig A). OGTT was repeated in 121 subjects after one year and a significant weight loss: GT changed from NGT to IGT in 7, and from IGT/T2DM to NGT in 24. New values of IS and IR were concordant with the new classes. (Fig B). These data indicate: 1) OGTT can be used to assess both IS and IR in different classes of GT, and for follow-up; 2) progressive and significant decreases of both IS and early IR are present in IGT and T2DM.



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INSULIN SECRETION AND INSULIN RESISTANCE IN IMPAIRED FASTING GLUCOSE AND IMPAIRED GLUCOSE TOLERANCE SUBJECTS

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Background and Aims: Several epidemiological studies have shown that ADA and WHO criteria differ in the diagnosis of diabetes and hyperglycemia: in particular impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), the intermediate categories of these two criteria, differ substantially. The aims of the present study was to compare the values of insulin secretion and insulin resistance, measured in 160 IFG (group A), 113 IGT (group B) and 122 IFG and IGT subjects (group C), with the values measured in a group of subjects with normal fasting glucose and normal glucose tolerance, on a oral glucose tolerance test (OGTT). **Materials and Methods:** Patients and controls, matched for sex, age and body mass index, were selected by a group of 1434 subjects (60% male, age 13-78 years), retrospectively evaluated, who were not known to be diabetics, who consecutively underwent a standard OGTT to establish their glucose tolerance. For all the subjects we calculated insulinogenic index ($\text{IRI}_{30-\text{fasting}}/\text{glucose}_{30-\text{fasting}}/\text{glucose}$) a index of insulin secretion and HOMAIR ($\text{fasting IRI}(\text{mU/ml}) \times \text{FBG}(\text{mmol/l})/22.5$) an index of insulin resistance. Serum insulin was measured by radioimmunoassay (INSI-CTK, Dia Sorrin Saluggia Italy). The intra assay coefficient of variation was $<4\%$ and the inter assay $<8.5\%$.

Results: Insulinogenic index was lower in A, B and C groups in comparison with control group (group A: 0.39 ± 0.30 , group B: 0.36 ± 0.26 , group C: 0.34 ± 0.26 , control group: 0.57 ± 0.70 , ANOVA $p < 0.01$), without statistically significant differences among the three groups of patients. HOMAIR was significantly higher in A, B and C groups in comparison with control group (group A: 2.95 ± 1.67 , group B: 2.95 ± 2.33 , group C: 3.42 ± 1.51 , control group: 2.24 ± 1.26 , ANOVA $p < 0.0001$), with a statistically significant difference between A and B groups in comparison with C group ($p < 0.02$ for both).

Conclusion: Data of the present study have demonstrated that both IFG and IGT patients presented modifications of insulin secretion and insulin resistance which characterize prediabetic conditions. In particular these modification are increased in patients who are IFG and IGT at the same time.

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Blood Flow in Heart and Brain

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Hyperinsulinaemia and insulin resistance are strong predictors of restenosis following coronary stenting.

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Background and Aims: Hyperinsulinaemia and diabetes mellitus are closely related to accelerated atherosclerosis. However, little is known about the importance of the metabolic alterations typical of Insulin Resistance Syndrome (IRS) in early restenosis after stenting in patients (pts) with coronary artery disease (CAD).

Materials and Methods: Seventy seven pts (mean age 57 ± 2 yrs and mean BMI 27.1 ± 0.5 kg/m²) were submitted to OGTT just before routine angiographic control after 6 months from coronary stenting. Pts had normal baseline glucose levels. IRS was defined as the presence of at least 3 of the following: hyperinsulinaemia, hypertriglyceridaemia, low HDL-cholesterol, visceral obesity, impaired glucose tolerance (IGT) or type 2 diabetes mellitus.

Results: IGT or type 2 diabetes mellitus were found in 43 pts, representing 56% of the whole population. Angiographic restenosis after stenting in multivessel CAD was found in 39 pts (51%). The presence of IGT and type 2 diabetes was not different in pts with or without restenosis while IRS was present in 15 pts with restenosis (38%) and in 8 pts without restenosis (21%). Moreover, pts with restenosis had increased HOMA levels (2.3 ± 0.2 vs 1.9 ± 0.1 , $p < 0.03$), fasting insulin (9.4 ± 0.7 vs 7.7 ± 0.3 μ U/mL, $p < 0.04$) and C-peptide levels (2.5 ± 0.1 vs 2.0 ± 0.1 ng/mL, $p < 0.003$). Interestingly, insulin response after OGTT was significantly increased in pts with restenosis after stenting. In particular, 30 min insulin levels were 66.5 ± 5.7 vs 49.2 ± 5.2 μ U/mL; $p < 0.03$ and 60 min insulin levels were 98.3 ± 11.2 vs 74.3 ± 5.6 μ U/mL; $p < 0.05$. Moreover, insulin incremental areas were 7631.3 ± 762 vs 5628.9 ± 423.8 μ U/mL (0-120 min); $p < 0.02$. At simple regression analysis, degree of restenosis at follow-up was positively correlated with fasting insulin ($r = 0.22$; $p = 0.08$) and C-peptide levels ($r = 0.29$; $p < 0.02$); insulin levels 30 min after OGTT ($r = 0.26$; $p < 0.04$), incremental insulin areas ($r = 0.27$; $p < 0.03$) and HOMA ($r = 0.30$; $p < 0.02$).

Conclusions: Hyperinsulinaemia and insulin resistance are main predictors of restenosis after stenting in patients with CAD.

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EFFECTS OF PREVIOUS RECURRENT HYPOGLYCAEMIA ON CLINICAL OUTCOME AND DISABILITY FOR STROKE IN DIABETIC PATIENTS.

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Background and Aims: Recurrent hypoglycaemia (RH) can appear as complication of intensive insulin treated type 2 diabetics. It has been described that RH could affect synaptic transmission, neuronal metabolism and neurotransmitters levels. The aim of the study was to investigate the effect that RH could have on clinical and functional disability for stroke in type 2 diabetic patients. **Materials and methods:** Three groups of insulin treated type 2 diabetics affected of acute large-vessel occlusive stroke were retrospectively analyzed. **Group 1:** Conventional insulin therapy, n=82, 49F,33M; aged 62.6 ± 12.3 yrs, mean known diabetes duration 10.3 ± 5.6 yrs. **Group 2:** Intensive insulin therapy without clinical history of hypoglycaemia, n=39, 20 F,19M; Aged 59.1 ± 10.3 yrs, mean diabetes duration 8.4 ± 6 yrs. **Group 3:** Intensive insulin therapy with clinical history of documented RH. n= 20, 9F,11M; Aged 60.1 ± 9.8 yrs, mean diabetes duration 9.1 ± 7 yrs. Clinical outcome in terms of neurological deficit was measured using Canadian Stroke Scale (CSS). Functional disability was measured using the modified Rankin scale (MRS). Data were collected at admittance after clinical stabilization, 6 and 12 months after stroke. HbA1c was determined at admittance. Data are show as mean \pm SD. Independent samples t-test was applied

Group	CSS 0	CSS 6m	CSS 12m	MRS 0	MRS 6m	MRS 12m	HbA1c
1	8.45 ± 1.23	9.06 ± 1.23	9.55 ± 0.86	1.82 ± 1.1	0.48 ± 0.87	0.24 ± 0.79	7.15 ± 1.4
2	7.85 ± 1.19	8.87 ± 0.96	9.48 ± 0.62	1.24 ± 0.91	0.38 ± 0.55	0.11 ± 0.4	5.85 ± 0.85
3	$6.82 \pm 1.1^*$	$7.81 \pm 1.43^{**}$	8.82 ± 1.17	$2.81 \pm 1.28^*$	$1.75 \pm 1.3^{**}$	$1.15 \pm 1.1^{**}$	$5.1 \pm 0.69^*$

* $p < 0.05$ group 1 vs group 3, ** $p < 0.05$ group 2 vs group 3

Results: Group 3 patients showed worse clinical outcome scores that reach statistical significance at admittance and 6 months that lose significance at time 12 months. In terms of disability, group 1 and 2 showed better functional scores at time 6 and 12 months that reach statistical significance. HbA1c was significantly lower in RH insulin treated diabetics as expected. **Conclusions:** Recurrent hypoglycaemia could induce biochemical and functional changes in central nervous system homeostasis that affect clinical and functional recovery after acute vascular injury and should be avoided.

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Secondary prevention of coronary heart disease in diabetic and non-diabetic patients in 9 European countries. Findings from EUROASPIRE I and II surveys

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Background and Aims: The aim of this study was to examine the trends in the clinical practice of secondary prevention of coronary heart disease (CHD) in diabetic and non-diabetic patients with reference to the therapeutic goals given in the 1998 Joint European Societies recommendations.

Materials and Methods: The EUROASPIRE I survey was undertaken in 1995-1996 and the EUROASPIRE II survey in 1999-2000. Nine countries participated in both surveys. Consecutive patients aged less than 71 years with the following diagnoses - coronary artery bypass grafting, coronary angioplasty, myocardial infarction, and acute myocardial ischaemia without infarction - were identified from hospital records and invited for an interview and examination at least 6 months after hospitalisation.

Results: In the EUROASPIRE I survey 641 (18.0%) of the 3,569 interviewed patients and in the EUROASPIRE II survey 740 (21.9%) of the 3,374 interviewed patients had previously diagnosed diabetes. Comparison of EUROASPIRE I and II data showed the following changes in the prevalence of lifestyles and risk factors among diabetic and non-diabetic patients: current smoking from 14.8% to 18.1% in diabetic and from 20.4% to 21.6% in non-diabetic patients; obesity (BMI ≥ 30 kg/m squared) from 34.2% to 44.4% in diabetic and from 23.3% to 29.5% in non-diabetic patients; raised blood pressure (systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg) from 64.5% to 60.3% in diabetic and from 53.4% to 52.1% in non-diabetic patients; and elevated total cholesterol (≥ 5 mmol/l) from 85.0% to 55.3% in diabetic and from 86.4% to 59.8% in non-diabetic patients. The use of cardiovascular drugs changed as follows: antiplatelet drugs from 81.8% to 82.2% in diabetic and from 81.0% to 84.3% in non-diabetic patients; betablockers from 52.0% to 66.0% in diabetic and from 54.0% to 66.5% in non-diabetic patients; ACE inhibitors from 43.2% to 52.0% in diabetic and from 26.5% to 40.0% in non-diabetic patients; calcium antagonists from 40.1% to 32.0% in diabetic and from 35.5% to 24.2% in non-diabetic patients; and lipid-lowering drugs from 28.6% to 64.0% in diabetic and from 32.8% to 62.6% in non-diabetic patients.

Conclusions: The adverse lifestyle trends and the unsatisfactory blood pressure and cholesterol control among both diabetic and non-diabetic CHD patients are a cause of concern. There is still a considerable potential in the lifestyle and risk factor management and some areas of prophylactic drug therapy among both diabetic and non-diabetic CHD patients.

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EFFECT OF DIABETES AND ROSIGLITAZONE ON MYOCARDIAL BLOOD FLOW.

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Background and Aims: Vascular dysfunction as characterized by abnormalities in vasomotor tone appears to be an important and early predictor of future coronary artery disease (CAD). Perhaps as a consequence of hyperglycemia, insulin resistance and/or dyslipidemia, this abnormality has been reported in individuals with DM2. Because of the complexity of invasive evaluations of coronary blood flow, in the past most studies of vasomotor function in diabetes have been performed in peripheral arteries. We therefore undertook to measure myocardial blood flow (MBF) and its response to adenosine (Ad) and cold pressor (CP) induced vasodilation using positron emission tomography (PET).

Material and Methods: PET scans of the heart have been performed at rest and during stress conditions in 16 men with type 2 diabetes (DM2) and 20 male healthy controls to date. DM2 were then treated with Rosiglitazone at 8 mg/day for 6 months and repeat PET scans performed. **Results:** Control subjects were without clinical or laboratory evidence of diabetes, hypertension, CAD or dyslipidemia. DM2 subjects were older, had significantly higher levels of fasting blood glucose (FPG) (221 ± 58 vs. 93 ± 12 , $p < 0.01$) and triglycerides (251 ± 104 vs. 134 ± 44 , $p < 0.05$). Only 2 DM2 subjects had either known CAD or evidence of lesions by PET. Resting MBF was higher in the subjects with DM2, reflecting differences in cardiac work and oxygen demand as indicated by their significantly greater heart rate-blood pressure product. However, resting MBF was similar when normalized to the rate-pressure product. In contrast, after Ad infusion, the increase in MBF or global myocardial reserve (GMR) was significantly greater in controls (4.73 ± 1.02 vs. 2.79 ± 1.05 mL/cc/min, $p < 0.001$) and remained higher even when normalized to the rate-pressure product. Differences in GMR were not explained by differences in age, lipid values or CAD prevalence; however, GMR was inversely correlated to FPG ($r = -0.65$, $p < 0.05$). Six DM2 subjects have completed rosiglitazone treatment to date and have had repeat PET scans during adenosine and CP conditions. In these individuals, MBF was increased compared to pre-treatment values during both ad ($P = 0.06$) and the CP test ($p < 0.05$) and approached levels seen in the healthy subjects. **Conclusions:** These data confirm that individuals with DM2 have abnormalities of MBF that appear in part related to endothelial dysfunction. Initial data suggests that treatment with an insulin sensitizer may dramatically improve these abnormalities of MBF. These studies also confirm that PET methodology may provide a noninvasive and quantitative approach to evaluating interventions designed to improve MBF.

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Insulin-induced increment of coronary flow reserve is dose-dependent.

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Background: We have recently demonstrated that insulin acts as a vasodilatory hormone also in the coronary vasculature. This action is shown to be endothelial derived and blunted in insulin resistance. However, the mechanisms and mode of action in coronaries are not known.

Methods: We quantitated myocardial blood flow, adenosine stimulated flow (adenosine 140 microg/kg/min) and flow reserve in 10 healthy men (age 33+/-7 years) using positron emission tomography and O-15-water. The measurements were performed in the fasting state, during physiological (1mU/kg/min) and high supraphysiological (5mU/kg/min) insulin infusions and normoglycaemic conditions.

Results: Physiological insulin infusion increased both the adenosine stimulated flow (from 3.63+/-1.61 to 4.41+/-1.76 mL/g/min, $p<0.05$) and the flow reserve (from 4.62+/-1.51 to 5.58+/-1.38, $p<0.01$). High insulin infusion was able to further enhance both the adenosine stimulated flow (to 5.05+/-1.92 mL/g/min, $p<0.05$) and the flow reserve (to 6.51+/-1.92, $p<0.01$). The effects could not be explained by the changes in systemic hemodynamics.

Conclusions: The results demonstrate that insulin acts as a vasoactive hormone in the coronary vasculature and that this effect is dose-dependent in healthy subjects.

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Information Technology

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CLOSED-LOOP GLUCOSE CONTROL WITH IV GLUCOSE SAMPLING AND SC INSULIN INFUSION: EVALUATION BY SIMULATION STUDIES

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Background and Aims: Fast acting insulin, improvements in continuous blood glucose monitoring and reliable insulin pumps have increased interest in the artificial pancreas. Extracorporeal wearable artificial pancreas is likely to adopt SC-SC route (subcutaneous (SC) glucose measurement and SC insulin infusion). We investigated a simpler IV(intravenous)-SC route using a computer simulator (SIM). **Materials and Methods:** We evaluated safety and efficacy of Model Predictive Control (MPC) algorithm, which drives SC infusion of insulin Lispro based on IV glucose values measured every 15min to achieve and maintain normoglycaemia at fasting conditions. SIM represents subjects with Type 1 diabetes, includes a model of glucose metabolism, and a model of subcutaneous absorption and kinetics of Lispro. Simulations involved 18 "synthetic" subjects, who were followed overnight from 10pm to 8am with starting plasma glucose of 11mmol/L. **Results:** Assuming 5% variation (VAR) in metabolic parameters, and a low to moderate measurement error (ERR) of 3, 5 and 8%, hypoglycemia (<3.3 mmol/L) occurred in one subject. Increasing ERR to 15%, six subjects recorded hypoglycemia, and in combination with 30% VAR eleven subjects presented hypoglycemia. Good efficacy was achieved with 5% VAR, and 3 to 8% ERR. Settling time, defined as the time to reduce plasma glucose from 11mmol/L to a target of 7.8mmol/L, was 197±11min (mean±SE). Average glucose from midnight and for the last 5 hours of the experiment was 6.4±0.1 mmol/L and 5.9±0.1mmol/L, respectively. AUC of plasma insulin between midnight and the end of the test was 9946±508mU/L.480min. **Conclusions:** Simulations demonstrate the ability of MPC to control plasma glucose in the presence of low to moderate ERR when adopting IV-SC route. The extent of VAR negatively influences the performance of MPC.

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Point of Care Testing and Connectivity: Benefits of data transfer from bedside to information systems.

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Background and Aims: Point of care testing is widely used in the healthcare environment. A major challenge has been integration of test results into host information systems such as Laboratory Information Systems (LIS), Hospital Information Systems (HIS) and Electronic Medical Records (EMR). Integration of POCT generated data into these systems is essential for several reasons: recording of and billing for reimbursement, managing data and measuring clinical outcomes as well as establishing and maintaining a quality POCT program. **Materials and Methods:** To encompass the variety of situations in which POCT devices are used, a number of connectivity solutions are required. From device to workstation, serial connections, modems or Ethernet connections are used to transmit data. From Workstation to LIS, scripted or (Electronic Data Interchange) EDI interfaces are essential. Outcomes from studies at several healthcare facilities that have implemented these solutions will be discussed. Outcomes measures include: improvements in compliance with quality control policies, improvements in compliance with policies regarding laboratory follow up on improvements in the ability to capture patient results for billing and reimbursement, improvement in the ability of the Point of Care Coordinator (POCC) to implement corrective actions for activities which require improvement and improvement in the quality of care for diabetic patients. **Results:** An example of the improvement in quality control compliance showed that the number of operators performing quality control at required intervals rose from an average of 71.5% to greater than 90% over a three month period. An example of workflow improvement is the savings of 6.6 to 9.2 hours per month downloading an average of 1349 to 10,511 test results per month. An example of the ability to capture patient results for billing demonstrated that 15.1% of bedside glucose results were not previously captured at a cost of \$36,000 annually. An example of the improvement in corrective actions is the reduction of the average time from occurrence of an error to contact with the nursing unit dropped from 36 days to 8 days. **Conclusions:** The power and flexibility of these systems allows healthcare facilities to optimize the application for their own unique needs.

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The Tayside Regional Diabetes Network - A Diabetes Managed Clinical Network for Tayside and Scotland

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Background and Aims: Clinical effectiveness can be undermined by insufficient or inappropriate access to information. Policies like the WHO health for all in the twenty-first century aim to promote population-based equitable care. Access to comprehensive information is an important part of this and the Tayside Regional Diabetes Network has been developed as a model for responding to this need. Its web site (www.diabetes-healthnet.ac.uk) includes all the most recent guidelines, patient information leaflets, comprehensive population-based patient data, research, news and educational resources.

Materials and Methods: With the support of all the principal patient carers in Tayside (Primary and Secondary Care) every possible source of patient information relating to diabetes is automatically linked and audited on a nightly basis. A variety of methods are utilised including manual validation via synchronised laptop computers and Optical Character Recognition (OCR) as not all information sources are electronic. The generic nature of the system means almost any source of data can be linked with the data being automatically quality assessed on importation. Guidelines, patient leaflets, educational resources and news are continually updated with these resources cross-referenced to the patient data. Security and confidentiality are addressed and all confidential information flows are encrypted. After extensive testing in pilot practices, the resource went live across Tayside Region (pop. ~400,000) on the 1st November 2000.

Results: Automated electronic record linkage has resulted in >1.5 Million records being held on 9,398 patients with known diabetes covering all aspects related to the condition from primary and secondary care. 73 out of 74 practices have requested access with the one remaining having no computers. This practice submits data via OCR forms and receives feedback via paper. 57% of practices and nearly all of secondary care use the system routinely and have performed 36,146 operations on patient data. There are 208 regular users of the system with > 500 users registered.

Conclusions: In the five months since the site went live, the region has embraced the system as the method for managing diabetes care. This is due to the comprehensive patient data, non-judgemental automated audit and the linked informational resources. The system has been subject to external review and is to play a key role in a diabetes system for Scotland. Expansion to other disease areas has already been started and the system is due for installation in two adjacent regions over the next three months. A live web demonstration can be given.

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TELEMEDICINE MAY ASSIST DIABETIC SUBJECTS TO IMPROVE METABOLIC OUTCOME AND PROMOTE PATIENT'S EMPOWERMENT.

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Background and Aims: The Optidiab Programme is a modular computerized system, designed as a comprehensive diabetes management system, covering for electronic documentation, assessment of care quality, and telemedicine. We have performed a pilot experiment to show the potential benefits of the telemedicine module (DiabTel) to promote the communication between patients and the diabetes team, to help patients in managing the disease on daily basis, and to increase patient's autonomy between regular visits to the Diabetes Unit. **Materials and Methods:** The system contains a Patient Unit (PU), portable microcomputer, designed for data collection (electronic patient logbook). Relevant events (SMBG, daily insulin therapy plan, dietary record) can be transferred through the public switched telephone line to the Medical Unit (MU), a PC-based tool, managing the information from patients and providing remote support to doctor's activities; it can operate as a 24-hour call center, and answers must be provided within 24 hours. Diabetes electronic medical record, data base for quality assessment and a smart card can also be optionally integrated in the system supporting doctor's activities. Ten DM-1 patients (8 females, 2 males), disease duration, 13.8 (6.5) years, volunteered to participate in a cross-over pilot study, divided in periods A (conventional) and B (experimental), randomly assigned, of 3 months duration each. Glycemic control was monitored (HbA1c, preprandial glycemia, frequency and severity of hypoglycemia), and quality of life evaluated with a specific questionnaire. SPSS package for Windows (v 8.0) has been used. Data are expressed as median (range); a Wilcoxon-rank test has been used for comparison (significance level, $p < 0.05$). **Results:** Number of therapeutic adjustments carried out by patients were similar in each period. HbA1c was 8.4% (6.9-9.1) at the beginning and 7.9% (6.6-8.9) at the end of the experimental period ($p = 0.053$), versus 8.1% (6.6-9.1) at the beginning and 8.2% (5.9-9.9) at the end of the control phase. No differences were observed between periods for the mean preprandial glycemia (178 mg/dL vs 170 mg/dL), and number of hypoglycemic episodes per week. Quality of life scores did not change. The system was well accepted by patients as a tool to improve metabolic control and communication with the medical team. **Conclusions:** This experience confirmed the feasibility of monitoring blood glucose levels, and the trend for metabolic improvement, with a telematic prototype, integrated into a diabetes management system.

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Construction of hospital-based datamart for the analysis of the clinical course of the diabetic patients

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Background and Aims: The prevalence of diabetes mellitus is very high, and many factors are involved in the development, response to the treatment, or the prognosis. In order to analyze this common and complex disease, a refined database will be a powerful tool.

Materials and Methods: Patients' fundamental information (sex, birth date and first visited date), information of prescriptions, and laboratory data were collected semi-automatically from the central data source of the hospital. Clinical history, family history and physical examination data (body weight and blood pressure) were collected manually from the individual's clinical record. Collected data were united as a datamart using a relational database (RDB). Requested data were selected with the structured query language (SQL) and analyzed statistically.

Results: About 14000 patients regularly visit our diabetes center at intervals of one to two months. 24296 patients, who have visited the center since 1997, were registered as study subjects. The number of total records put in the datamart were more than 600000, which consist of 307347 prescriptions, 110725 laboratory data (mainly HbA1c), 62807 urinary analyses, 16593 diagnoses, 15941 physical examinations, 7128 glucose or meal tolerance tests, 2734 inpatients' information, 700 family histories etc. Characteristics of the patients at the first visit were 57.0% males with mean age 50.6yrs, BMI 23.7, BP 140/85 and HbA1c 8.9%. For the first step to utilize the datamart, we analyzed the HbA1c profiles. For the patients followed at the outpatient clinic, HbA1c levels (%) were 8.8 (0 month), 7.1 (3mo), 7.1 (6mo), 7.2 (9mo) and 7.2 (12mo) after the first visit. For the patients admitted to the hospital within 3 months after the first visit (mean stay 19.8 days), HbA1c levels were 9.1 (0mo), 6.8 (3mo), 6.9 (6mo), 6.9 (9mo) and 7.1 (12mo) after the admission. These results shows that HbA1c profiles of patients admitted to the hospital were marginally better than those of non-admitted patients. On the other hand, HbA1c profiles of the patients, who admitted to the hospital after more than one year's follow-up at the outpatient clinic (mean stay 16.8 days), were 8.0 (0mo), 7.3 (3mo), 7.4 (6mo), 7.5 (9mo) and 7.6 (12mo). Improvement of HbA1c was small and rapidly deteriorated again in these patients.

Conclusions: We constructed the datamart of the patients who visited our diabetes center during the past four years. Datamart (or data warehouse) is very useful to analyze a large quantity of data in a short period. When the datamart is complete, a lot of useful information will be available using multi-dimensional analysis or data-mining methods.

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Clinical Issues in Diabetes Practice

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CASE REPORT: AMPHETAMINE-INDUCED KETOALKALOSIS?

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Presentation: A 26 year old male was admitted with a four day history of vomiting without abdominal pain, distension or diarrhoea. He had an 11 year history of type 1 diabetes (most recent HbA1c 8.6%), but was otherwise free from microvascular complications. He was taking human Mixtard 30; 40 units twice daily. **On admission:** He was lethargic but with a Glasgow Coma Score of 15. He was tachypnoeic and dehydrated, but normotensive, and general examination was unremarkable. Investigations suggested ketoacidosis with a capillary glucose >33 mmol/l, accompanied by heavy ketonuria and glycosuria. However, his arterial blood gases on air showed a metabolic alkalosis with pH 7.69, pCO₂ 4.6 kPa, pO₂ 12.8 kPa, [HCO₃⁻] 41.1 mmol/l. Further tests revealed [Na⁺] 123 mmol/l, [K⁺] 4.2 mmol/l, urea 22.4 mmol/l, glucose 38.3 mmol/l and random cortisol 964 nmol/l. **Treatment and Progress:** He received intravenous fluids with potassium supplementation, and intravenous insulin. Vomiting settled within 24 hours, whilst his electrolytes normalised within 72 hours. Gastroscopy revealed oesophagitis and duodenal ulceration, but no gastric outflow obstruction. On further questioning he denied antacid ingestion. The probable cause of his metabolic upset became apparent when he disclosed that his vomiting began following his first recreational use of amphetamine. **Summary:** This patient presented with dehydration and ketonaemia in the presence of a marked metabolic alkalosis. We suggest that amphetamine ingestion could lead to increased central dopamine release, causing profound and prolonged vomiting with loss of [H⁺] ions, and consequent alkalosis, sufficient to protect against acidosis in the presence of ketonaemia. Diabetic ketoalkalosis is rarely reported and has not been described in relation to recreational drug use.

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Diabetes as Risk Factor for Incidence and Location of Colon Cancer

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Background and Aims: The aim of our study was to determine, on the basis of analysing data from the General Health Insurance Office in 3 independent areas of the Czech Republic (Pilsen -North - agriculturally industrial, Strakonice - agricultural, Sokolov - industrial), the relation between prevalence of colorectal carcinoma and diabetes mellitus. The population of these 3 areas in the Czech Republic represented in 237 943 persons.

Materials and Methods: Since the causality of the relation between diabetes and colorectal carcinoma is not clear, the main factor to be considered was the ODDs ratio. The prevalence of diabetes was 5.67% (85.3% without insulin). All patients with medical procedure performed during one year for colon cancer (ICD diagnosis C18,C19,C20, C21) and for diabetes were included. It was not possible to analyse the type of diabetes, but it was possible to differentiate insulin treated IDDM and insulin non treated NIDDM diabetes.

Results: The results are divided to four groups: I- Total all colon cancer, II. Ca colon-diagnosis C18, III. Rectosigmoidal cancer- diagnosis C19, IV. Rectal and anal cancer-diagnosis C20+C21.

I. Ca colon + rectosigmoidum + rectum (C18-C21): ODDs ratio- Total 3.15 DM males 2.93, DM females 3.27, IDDM 3.74, NIDDM 2.99 (p= <0.0001, <0.0001, <0.0001, <0.0001, <0.0001)

II. Ca colon (C18) ODDs ratio- Total 2.36, DM Males 2.12, DM females 2.39, IDDM 1.98, NIDDM 2.37 (p= <0.0041, <0.0179, <0.0060, <0.1194, <0.0201).

III. Ca rectosigmoidum (C19): ODDs ratio- Total 3.68, DM Males 3.25, DM females 4.40, IDDM 3.18, NIDDM 4.04 (p= <0.0001, <0.0001, <0.0001, <0.0001, <0.0001)

IV. Ca rectal + anal part (C20+C21): ODDs ratio: Total 4.21, DM Males 3.42, DM females 5.42, IDDM 3.55, NIDDM 4.26 (p= <0.0001, <0.0001, <0.0001, <0.0001, <0.0001).

Conclusions: 1. The high risk of colorectal cancer in diabetes was found - ODDs ratio from 2.12 to 5.42 shows diabetes as a very high risk factor for colon cancer. 2. Highest relation was found between rectal cancer and diabetes. 3. According to the increasing risk to the distant part of gastrointestinal track we suppose that dietary factors can influence the risk. 4. The risk is higher in patients without insulin treatment. 5. Both, the incidence of diabetes and colorectal cancer, are increasing. It seems that this relationship can be causal.

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LOWER RISK OF HYPOGLYCAEMIA WITH REPAGLINIDE COMPARED TO GLIBENCLAMIDE DURING RAMADAN FASTING IN MUSLIM PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Background and Aims: Adult Muslims observe the month of Ramadan by fasting from sunrise to sunset. Diabetes management of Muslim patients during this period is a challenge as long fasting periods between two large meals incur a risk of hypoglycaemia while on sulphonylurea or insulin treatment. In a 14-week open label, parallel group trial, the glycaemic control in Muslim type 2 diabetic patients (practicing Ramadan fasting) treated with a prandial glucose regulator (repaglinide, REP) was compared with that of a sulphonylurea (glibenclamide, GLIB).

Materials and Methods: The study was conducted in five countries: Malaysia, United Kingdom, France, Saudi Arabia and Morocco. A total of 235 patients previously treated with a sulphonylurea were randomised to receive either REP (n = 116) preprandially 3x daily or GLIB (n = 119) preprandially 1x/2x daily, 6 weeks before the Ramadan. During the 4-week Ramadan, patients changed their eating pattern to the two meals daily, with little change in energy intake, while the total daily dose of REP was re-distributed to two preprandial doses. After the Ramadan, patients resumed their pre-fasting meal and treatment routines for another 4 weeks.

Results: During Ramadan, a statistically significant reduction in mean serum fructosamine concentration was seen in the REP group (-3.8%, p <0.05) but not in the GLIB group (-0.8%, ns), compared to baseline values. Ninety-three REP-treated patients recorded 2228 readings of mid-day blood glucose (BG) concentrations, and 100 GLIB-treated patients recorded 2361 such readings. The number of events with mid-day BG <4.5 mmol/l (indicative of risk of hypoglycaemia) was significantly lower with REP (n=62, 2.8%) than GLIB (n=187, 7.9%) (p = 0.001). Among those with hypoglycaemic episodes (BG <2.8 mmol/l), 3 episodes were reported by REP-treated patients (0.03 events/patient/month) compared to 6 episodes by GLIB-treated patients (0.05 events/patient/month), between-group difference, ns. Apart from hypoglycaemia, both treatments were equally well tolerated - with no significant difference in adverse events or other clinical safety parameters.

Conclusions: During Ramadan, Muslim type 2 diabetes using prandial REP showed a trend towards better glycaemic control, and had significantly lower risk and lower frequency of hypoglycaemia than patients using GLIB.

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ANTIPSYCHOTIC DRUGS MAY WORSEN METABOLIC CONTROL IN TYPE 2 DIABETES

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Background and Aims: Several studies indicate that diabetes mellitus type 2 is more common among schizophrenic patients than in the general population. Also, it is suggested that - especially atypical - antipsychotic drugs can induce diabetes. The aim of the study was to assess the association between the use of antipsychotic drugs and alterations of glycaemic control.

Materials and Methods: Pharmacy dispensing records and hospital discharge data from 1991-1999 were obtained from the PHARMO system, comprising about 320,000 patient histories. Onset of diabetes was defined as date of first prescription of an oral hypoglycaemic agent. We performed a nested case-control study, including patients who had at least two years medication history after diagnosis. Switching from oral hypoglycaemic agents to insulin therapy was considered a proxy for deterioration of β-cell function. Cases were subjects converted to insulin within this two-year interval, controls were subjects still on oral therapy. We compared the use of antipsychotic drugs between cases and controls.

Results: We identified 3,104 newly diagnosed patients with type 2 diabetes of which 1,503 (49.3%) were men; mean age at onset was 63.5 ± 13.3 years, mean duration of diabetes 3.9 ± 2.0 years and eventually 19.4% switched to insulin therapy. Of the entire study population, 2,558 patients had at least two years of medication history and 199 (7.8%) cases were identified. Patients using antipsychotics were more likely to switch (15.4% versus 7.5% in non-users, OR: 2.24, 95% CI 1.25-4.04). Adjustment for age at onset and gender did not change the results; ORs were 2.27 (1.25-4.12) and 2.24 (1.24-4.06), respectively. Atypical antipsychotic drugs did not appear to affect β-cell function more markedly than classical drugs.

Conclusions: It seems that use of antipsychotics in type 2 diabetes mellitus is associated with switching to insulin therapy (i.e. 'secondary failure'), especially in the first two years of the disease.

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NON-ISLET CELL TUMOUR HYPOGLYCAEMIA IN A PATIENT WITH A METASTATIC LEYDIG CELL TUMOUR

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Non-Islet Cell Tumour Hypoglycaemia (NICTH) is a rare cause of spontaneous hypoglycaemia. We report a case of NICTH associated with a metastatic Leydig cell tumour.

A 75 year old man presented to the casualty department of our hospital with profound spontaneous hypoglycaemia (venous glucose of 0.7 mmol/l). Two years ago he was found to have Leydig cell tumour of his right testicle and had an orchidectomy. The tumour was malignant with vascular invasion and widespread metastases in inguinal and pelvic lymph nodes. He was treated with palliative radiotherapy to his right groin but unfortunately there was no significant response. The patient declined to have palliative chemotherapy in view of the anticipated side effects. Lymphadenopathy progressed relentlessly in the inguinal, pelvic and retroperitoneal region. Two years from diagnosis he presented with spontaneous hypoglycaemia and was revived with 10% dextrose. He looked very cachectic; vital parameters were stable, cardiovascular and respiratory systems were normal and there was no focal neurology. He had a large, irregular, hard mass of lymph nodes in his abdomen. Apart from the blood glucose, biochemistry was within the normal reference range, including liver function. Cortisol was 928 nmol/l. Growth hormone was 2mU/L. Serum insulin was < 3mmol/l and C-peptide was undetectable. Insulin like growth factor I (IGF I) was 9 nmol/l and IGF II was 118.2nmol/l; the ratio of IGF II/IGF I was 13.1 (a ratio of > 10 is diagnostic of NICTH). He received a continuous 10% dextrose infusion and subsequently, he was given dexamethasone and human recombinant Growth Hormone (hGH). Hypoglycaemia improved enabling us to stop his dextrose infusion. Unfortunately, two weeks later he died.

This is the first reported case of NICTH associated with Leydig cell tumour.

OP 40 Exocytosis

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Two-photon excitation imaging of insulin exocytosis from intact pancreatic islet of Langerhans.

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Background: Cyclic AMP is considered a competence factor for insulin exocytosis, and its antagonism by Rp-cAMP markedly inhibits exocytosis in single pancreatic β cells. In contrast, studies with whole-islet preparations have detected only small effects of cyclic AMP antagonists on glucose-induced insulin secretion as measured by radioimmunoassay. **Aims:** To resolve the discrepancy between the results obtained with single cells or whole islets, we have applied a new imaging technique that is based on two-photon excitation of fluorescent polar tracers and which allows visualization of insulin exocytotic events in intact islets. **Materials and Methods:** Pancreatic islets were isolated from mice and were immersed in a solution containing the polar fluorescent tracers, sulforhodamine B. The tracers were excited by laser-scanning microscopy with a mode-locked femtosecond-pulse Ti:sapphire laser. Images were acquired from relatively superficial planes within the islets. **Results:** Immersion of islets in a solution containing a polar tracer resulted in retrograde labeling of microvessels and interstitial space surrounding β cells. Exposure of islets to high glucose (20 mM) induced the rapid appearance of fluorescent spots with a diameter of 0.3 micrometer, consistent with the size of insulin granules, at the surface of β cells; these spots decayed with time constants between 1 and 20 s. This phenomenon can be interpreted to represent the rapid filling of insulin vesicles by the polar tracer and the subsequent flattening of vesicles fused with the plasma membrane. The fluorescent spots were detected predominantly at the surface region of β cells not directly facing microvessels. The exocytotic events were regulated by the cytosolic Ca^{2+} concentration and occurred in two phases, an initial transient phase and a subsequent sustained phase. Rp-cAMP prevented the first phase of insulin exocytosis, without markedly affecting Ca^{2+} signaling. In contrast, forskolin, which activates adenylate cyclase, augmented exocytosis in both phases. We also observed that membrane-permeable lipophilic compounds tended to be trapped in the superficial cell layers of islets, which may account for the smaller effects of cyclic AMP antagonists on insulin secretion from whole islets as measured by radioimmunoassay. **Conclusions:** We have succeeded in visualizing insulin exocytosis in intact pancreatic islets by two-photon excitation imaging. This approach clarified that the first phase of glucose-induced insulin secretion is especially sensitive to cAMP, consistent with the potent effects of cAMP on the fast component of exocytosis in single β cells.

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Delay between fusion pore opening and peptide release from insulin-containing granules in Ins-1 cells

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Background and Aims: Insulin-release is preceded by recruitment, docking at the plasma membrane, and priming of secretory granules for release. This is reflected by the kinetic properties of exocytosis, which suggest that granules exist in functionally distinct pools. Only a fraction of the cell's granules, referred to as the readily releasable pool (RRP), is available for instant exocytosis, but it is not clear how RRP relates to granules that are physically docked at the plasma membrane. In addition, little is known about how peptides are released from the granule during exocytosis. **Materials and Methods:** Exocytotic membrane fusion and cargo release were studied in insulin-secreting Ins1-cells by capacitance measurements and simultaneous confocal imaging of secretory granules tagged with a soluble amylin-EGFP fusion protein.

Results: Labelled granules with varying degrees of mobility were seen throughout the cell interior, while immobile granules were primarily found at the plasma membrane. During voltage-clamp depolarizations, single exocytotic events could be detected by time-resolved confocal microscopy as sudden disappearance of the immobile, plasma membrane-associated granule fluorescence. Strong stimulation with trains of voltage-clamp depolarizations to deplete the readily releasable pool (RRP) correlated with visible exocytosis of 10-20% of the docked granules. Some remaining granules could be released after RRP-recovery, while new granules rarely appeared. Fusion pore opening (seen as granular pH equilibration and quenching of EGFP-fluorescence) occurred ~0.3 s after the capacitance increase ($t=85$ ms), and peptide release was delayed another 1.6 s.

Conclusions: We propose that: a) RRP is a subset of the docked pool; b) RRP-refilling involves chemical modification of granules already in place; c) efflux of the hormone through the fusion pore does not contribute to secretion; and d) the fusion pore must dilate significantly to allow peptide release. The delay observed for the latter may allow regulation of insulin-release after exocytosis of the granule.

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CYCLIC AMP STIMULATES INSULIN EXOCYTOSIS BY MODIFICATION OF GRANULES ALREADY PRESENT AT THE RELEASE SITES

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Background and Aims: Increasing evidence suggests the importance of cAMP-signalling for biphasic insulin secretion in vivo. cAMP potentiates insulin exocytosis by two mechanisms: a) the readily releasable pool (RRP) of granules increases by a mechanism not requiring protein kinase A (PKA), b) PKA-dependent acceleration of granule recruitment to RRP. Recently, it was suggested that the cAMP-binding protein cAMP-GEFII via interaction with the rab-protein RIM2, is responsible for the former action. Here we have investigated how physical granule translocation contributes to the cAMP-stimulation of exocytosis.

Materials and Methods: Capacitance measurements of exocytosis were combined with confocal imaging of insulin granule motion in INS-1 or mouse B-cells. Insulin granules were probed using LysoTrackerRed or by transfection of EGFP-Phogrin. RIM2 expression was perturbed by anti-sense treatment.

Results: The effects of cAMP on INS-1 cell exocytosis were very similar to those reported in mouse B-cells. Exocytosis elicited by trains of 10 voltage-clamp depolarisations resulted in biphasic exocytosis. During the first pulse, which corresponded to discharge of RRP, exocytosis was >5-fold faster than during the remainder of the train. Forskolin increased both the RRP (17 ± 3 & 85 ± 8 fF, before & after addition of forskolin; $P < 0.001$; $n = 14$) as well as recruitment of additional granules during the train stimulus (99 ± 12 & 291 ± 38 fF, before & after addition of forskolin; $P < 0.01$; $n = 14$). In mouse B-cells, anti-sense treatment verified the importance of RIM2 for the PKA-independent stimulation of RRP by cAMP ($P < 0.05$; 89 ± 15 & 11 ± 10 fF; $n = 21$ and 24, sense & anti-sense). In parallel recordings of granule movement and exocytosis in INS-1 cells, forskolin stimulated exocytosis within <2 minutes. Granule movements consisted of 1) rapid microtubule-guided saltatory jumps (velocities 200-2000 nm/s), 2) random diffusion, and 3) slow directed movements (velocities 20-40 nm/s) associated with the peripheral actin network. Only the latter type was stimulated by forskolin (30 ± 6 and 39 ± 4 fF, before & after addition of forskolin; $P < 0.05$; $n = 14$). Interestingly, the effect was only apparent at times later than 4 min after addition of forskolin, was strictly dependent on PKA and was abolished by 8-Br-Rp-CAMPS.

Conclusions: 1) Cyclic AMP stimulates exocytosis primarily by modifying insulin granules situated at the plasma membrane. 2) Sustained stimulation reveals a PKA-dependent increase in peripheral granule motion. 3) RIM2 mediates the cAMP-induced increase in RRP in mouse B-cells.

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SNAP-25 AND SYNTAXIN INFLUENCE THE READILY RELEASABLE POOL OF INSULIN-CONTAINING GRANULES IN MOUSE PANCREATIC B-CELLS

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Background and Aims: The secretory granules within the pancreatic B-cells must be docked and primed prior to fusion and release. In many endocrine cells as well as the pancreatic B-cell SNARE-proteins have been suggested to be present in these processes. The aim of this study was to investigate the involvement of the t-SNARE proteins SNAP-25 and syntaxin in the exocytotic process of primary mouse B-cells.

Material and Methods: For this purpose we used capacitance measurements to investigate the temporal aspects of exocytosis on a single cell level. The standard whole-cell configuration of the patch-clamp technique was used which allows an intracellular application of the different antibodies used.

Results: The presence of both SNAP-25 and syntaxin was confirmed using Western Blot analysis and the distribution of the proteins within the B-cell was investigated by immunostaining prior to the functional studies. A train of ten 500 ms depolarising pulses from -70 mV to 0 were applied to single B-cells. The two first pulses of the train were used to estimate the size of the readily releasable pool (RRP) whereas the following pulses represent mobilisation of granules from a reserve pool. Under control conditions the RRP could be estimated to contain 66 ± 13 granules ($n = 15$), using a conversion factor of 2 fF/granule. It was diminished to 18 ± 9 granules ($n = 5$) in the presence of an antibody against SNAP-25. This antibody also reduced the increase in membrane capacitance during the latter pulses of the train to 34 ± 19 fF ($n = 7$) compared with 169 ± 26 fF ($n = 20$) under control conditions. In the presence of an antibody against syntaxin mobilisation was reduced, and the RRP contained 23 ± 7 granules, which is a similar result observed with blocking SNAP-25.

Conclusions: The data suggests that SNAP-25 and syntaxin are crucial for the number of granules within the RRP. Since these granules is proposed to be released during the first phase of insulin secretion, lack of functional t-SNARE-proteins might cause a reduced first phase appearing in patients with type-2 diabetes.

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INVOLVEMENT OF THE SMALL G-PROTEIN RAC1 IN GLUCOSE AND FORSKOLIN INDUCED INSULIN SECRETION IN ISLET (INS-1) β -CELLS

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Background and Aims: In addition to its role in regulating cytoskeleton organization, the monomeric GTP-binding protein Rac (a member of Rho family GTPase) may be also implicated in exocytotic secretion. This work studied the possible involvement of Rac1 in insulin secretion. **Materials and Methods:** Islet β -cells (INS-1) were stably-transfected with c-myc-tagged dominant-negative (N17) or dominant-positive (V12) Rac1 mutants, with empty vector transfected cells as control. Insulin secretion from these cells was assayed by radioimmunoassay. Cellular F-actin changes were assessed by confocal microscopy after rhodamin-phalloidin staining and quantitated following extraction of the dye. Localization of mutant Rac1 was examined by subcellular fractionation followed by immunoblotting using anti-c-myc antibody. **Results:** The increment of insulin release stimulated by 15 mM glucose plus 1 μ M forskolin (raising cAMP) was markedly reduced by ~49% ($n = 6$, $p < 0.01$) in dominant-negative Rac1(N17) transfected cells. Insulin secretion induced by 15 mM glucose alone was slightly but significantly decreased by ~15% ($n = 6$, $p < 0.05$) while high K^+ (34 mM)-stimulated secretion was not altered in Rac1(N17) transfected cells. Transfection with dominant-positive Rac1(V12) did not affect stimulated insulin release by above secretagogues. Cells with Rac1(N17) tended to rounded up and growth in colony while Rac1(V12) transfected cells revealed decreased sharp filopodia. Phalloidin staining of F-actin web underneath plasma membrane was weakened in Rac1(V12) transfected cells and almost disappeared in cells with Rac1(N17). F-actin bundles disappeared and F-actin contents were decreased in both Rac1(N17) and Rac1(V12) transfected cells. Dominant-negative Rac1 was expressed only in mitochondrion and insulin granule fractions while the dominant-positive Rac1 was localized in both cytosol and other fractions including microsomes. **Conclusions:** Expression of dominant-negative or -positive Rac1 altered INS-1 cell morphology and interfered with F-actin organization. Dominant-negative Rac1 affected glucose and forskolin induced insulin secretion, indicating that Rac1 may also play a role in the regulated secretion probably beyond acting on cytoskeleton.

OP 41

Fat Cell Hormones

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A diet enriched in fat induces increased expression of resistin and insulin resistance in lean rats.

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Background and Aims: It was recently demonstrated that insulin resistance in obese, fat fed mice is related to increased secretion of the fat cell hormone resistin. To investigate the possible role of resistin for dietary induced insulin resistance in lean animals, we studied the expression of resistin mRNA in fat tissue from lean rats rendered obese by a high fat diet.

Materials and Methods: Twelve male Wistar rats (200-250g) fed a diet with 60% lard (HF) gained 42g and 12 control rats fed a standard chow with 5% fat (CT) gained 142g in weight during 7 weeks. Fasting levels of glucose and insulin were not different between groups. Thirty minutes after an intraperitoneal injection of a mixture of insulin (100 mU/kg) and glucose (2g/kg), the insulin levels were elevated but not different between groups, whereas the glucose levels in HF rats were significantly elevated compared to CT. Fat pad weight was lower in HF rats. The expression of resistin mRNA was quantified by means of a ³²P-labelled probe in Northern blots of total RNA isolated from fat pads.

Results: Resistin mRNA expression was markedly higher in HF rats than in CT rats (146±9 vs 32±7 mean ± SE, arbitrary units, p<0.001).

Conclusions: Increased expression of resistin can be induced by a high fat diet in spite of a reduction in fat mass. The hormone resistin is likely to mediate the dietary induced, whole-body insulin resistance in this condition.

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Free Leptin, Bound Leptin And Soluble Leptin Receptor In Type 2 Diabetes And In The Polycystic Ovary Syndrome.

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Background: Both type 2 diabetes and the polycystic ovary syndrome (PCO) are characterised insulin resistance. Free leptin (FL) levels are related to fat mass and increased by insulin, but the relationship of other components of the leptin system [bound leptin (BL) and soluble leptin receptor (SR)] to insulin and glycaemic control has not been studied so far. **Material and Methods:** We measured fasting FL, BL, SR, insulin and HbA1c in 31 premenopausal women with type 2 diabetes age (mean±SD) 39±7.6 years, BMI: 31.1±7.37 kg/m²; and in 20 non-diabetic women with the PCO syndrome: age 27.1±7.6 years, BMI 38.0±8.9 kg/m². Correlation analysis was performed by a univariate model. **Results:** In the diabetic group there was a correlation between FL and BMI (r=0.43, p=0.01) and a trend towards a positive correlation with fasting insulin (p=0.12), and HbA1c (p=0.10). There was no correlation between BL and BMI or insulin. SR correlated positively with BMI (r=0.36, p=0.05) and HbA1c (r=0.41, p=0.02). There was no correlation between SR and fasting insulin. In the PCO group there was a strong correlation between FL and BMI and fasting insulin (respectively: r=0.48, p=0.03, r=0.55, p=0.01). There was no significant correlation between BL and fasting insulin, or between SR and the BMI, but in contrast to diabetic group, there was a negative correlation between SR and fasting insulin (r=-0.53, p=0.01). **Conclusions:** In all women free leptin correlates with the body mass index, but the correlation between free leptin and fasting insulin is stronger in non-diabetic subjects. Soluble leptin receptor concentrations correlate positively with body mass index and HbA1c in diabetic women, while in non-diabetic subjects there is a negative correlation with fasting insulin. It remains to be established whether insulin can influence soluble leptin receptor concentrations and whether high soluble leptin receptor levels can be used as a marker of poor diabetic control.

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DIRECT REGULATION OF LEPTIN SECRETION BY SATURATED, POLYUNSATURATED AND MONOUNSATURATED FATTY ACIDS IN CONTROL AND INSULIN-RESISTANT RAT ADIPOCYTES.

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The regulation of leptin secretion by different fatty acids is not well established. The aim of this study was to determine whether saturated fatty acids (palmitic acid), n-3 polyunsaturated fatty acids (docosahexaenoic: DHA and eicosapentaenoic EPA acids) and monounsaturated fatty acids (oleic acid) have differential effects on leptin secretion in adipocytes of insulin-resistant sucrose-fed rats or in control rat adipocytes. **Materials and Methods:** Twenty male Sprague-Dawley rats were randomized into two groups: the control group (C, n=10) was submitted to a standard diet and the sucrose group (SC, n=10) was submitted to a sucrose enriched diet (57% sucrose). After 3 week diet, epididymal adipocytes were isolated and incubated two hours with either insulin (100 ng/ml) or insulin associated with the different fatty acids (0.5 mM). **Results:** After two hours incubation, leptin secretion was increased by insulin in control rat adipocytes, this insulin action was majored by EPA (p<0.05) and inhibited by palmitic acid (p<0.05). In sucrose-fed rat adipocytes leptin secretion was enhanced by insulin too (p<0.05), this insulin stimulation was not altered in association with EPA but was lowered by the other fatty acids (palmitic acid: p<0.05, DHA: p<0.05 and oleic acid: p<0.05). **Conclusion:** Fatty acids in presence of insulin make a direct action on leptin secretion by adipocytes. This action is dependent on the fatty acid unsaturation degrees and on the adipocytes origine (isolated either from insulin-resistant rats or from control rats). Fatty acids have direct and differential effects on leptin secretion regulation, this would be an additional data in the understanding of the beneficial action of n-3 polyunsaturated fatty acids on metabolic diseases.

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Quantitative expression of leptin receptor signalling components in the pituitary and pancreas of diet-induced obese and genetically obese ob/ob mice

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Background and Aims: In addition to its hypothalamic actions, there is now much evidence supporting a significant role for leptin in other tissues. The functional form of the leptin receptor, OB-Rb, is expressed in the pituitary and pancreas amongst other tissues. Leptin binding to OB-Rb activates the JAK-STAT pathway and thereby elevates expression of inhibitors of STAT signalling e.g. SOCS-3 and CIS. The aim of this study was to analyse expression of genes involved in leptin receptor-mediated signalling in the pituitary and pancreas of centrally leptin-sensitive (ob/ob) and leptin-insensitive (dietary obese) mouse models of obesity.

Materials and Methods: The fluorogenic TaqMan method of quantitative RT-PCR was used to measure changes in mRNA expression of the short leptin receptor isoform OB-Ra, OB-Rb, STAT3, STAT5, SOCS-3 and CIS. Expression was analysed in the pituitary and pancreas of AKR/J mice fed on either a chow or a palatable diet (DIO) for 14 weeks, and in C57BL/6 lean and leptin-deficient C57BL/6 ob/ob mice. Statistical analysis was performed by analysis of variance (ANOVA) between the groups using the housekeeper gene cyclophilin as a covariate.

Results: Plasma leptin levels were 7.27 ± 1.51 ng/ml in chow-fed AKR/J mice, 117 ± 49.39 ng/ml in DIO mice, 3.32 ± 0.67 ng/ml in C57BL/6 lean mice and not detectable in C57BL/6 ob/ob mice. In the pituitary, OB-Rb, STAT3, STAT5 and SOCS-3 mRNA levels were reduced in ob/ob mice compared to lean controls by 55%, 41%, 61% and 53% (P<0.05), respectively, but no changes were observed in DIO mice compared to chow fed controls. OB-Ra, OB-Rb, STAT5, and SOCS-3 were higher in AKR/J lean mice compared to C57BL/6 lean mice by 106, 67, 108 and 136% (P<0.05), respectively. In the pancreas of ob/ob mice there was a 26% increase in STAT5 expression and a 36% increase in SOCS-3 expression (P<0.05), but again there were no changes in DIO compared to chow-fed mice. Also, in the pancreas of AKR/J lean compared to C57BL/6 lean mice there was an increase in STAT3, STAT5 and CIS expression of 31, 35 and 120% (P<0.05), respectively.

Conclusions: The leptin system is upregulated in AKR/J compared to C57BL/6 lean mice. The expression of genes involved in leptin signalling was generally reduced in the pituitary but slightly increased in the pancreas of ob/ob mice relative to lean controls, indicating that leptin sensitivity may be regulated in a tissue-specific manner.

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LEPTIN-DEPENDENT INHIBITION OF HEPATIC GLUCONEOGENESIS IS MEDIATED BY INSULIN RECEPTOR SUBSTRATE-2

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Background and Aims: Leptin may exert metabolic effects which could result from reduction in food intake mediated by the hypothalamus and/or from binding to its peripheral receptors. Therefore, we examined the direct effects of leptin (0.5, 5 and 25 nM; vs. control, CON) in isolated perfused livers of 20 h fasted rats (90 min, n=4-6/group). **Materials and Methods:** Glucose production, lactate uptake, bile flow, and portal pressure were continuously monitored. Phosphoenolpyruvate carboxykinase (PEPCK) activity was determined in total liver cytosol using the $\text{NaH}^{14}\text{CO}_3$ fixation assay. For measurement of tyrosine phosphorylation (pY), the insulin receptor (IR) and the insulin receptor substrates (IRS) 1 and 2 were immunoprecipitated from liver cytosol and then immunoblotted for phosphotyrosine using chemiluminescent detection. Immunoprecipitated IRS-1/2 was immunoblotted for associated p85-kD subunit of phosphatidylinositol 3-kinase (PI-3K) and tested for associated PI-3K activity by immune complex kinase assay using radiolabeled phosphatidylinositol and thin layer chromatography/autoradiography. The efficacy of the immunoprecipitation of IR and IRS-1/2 was controlled by homologous immunoblotting. **Results:** Leptin reduced hepatic glucose production (0.5 nM: 0.13 ± 0.01 , 5 nM: 0.14 ± 0.02 , 25 nM: 0.23 ± 0.04 ; $P < 0.001$ vs. CON: $0.38 \pm 0.04 \mu\text{mol min}^{-1} \text{g liver}^{-1}$) and PEPCK activity (0.5 nM: $50 \pm 1\%$; 5 nM: $57 \pm 2\%$; 25 nM: $79 \pm 2\%$; $P < 0.001$ vs. CON). Leptin increased IR pY slightly (0.5 nM: $120 \pm 3\%$, 5 nM: $113 \pm 3\%$, $P < 0.01$ vs. CON). In dose-dependent fashion, leptin reduced IRS-1 associated PI-3K (0.5 nM: $70 \pm 9\%$, 5 nM: $38 \pm 2\%$, 25 nM: $16 \pm 1\%$; $P < 0.006$ vs. CON) and its activity (0.5 nM: $68 \pm 4\%$, 5 nM: $24 \pm 1\%$, 25 nM: $10 \pm 1\%$; $P < 0.001$ vs. CON). In contrast, leptin stimulated IRS-2 pY (0.5 nM: $570 \pm 36\%$, 5 nM: $350 \pm 43\%$; $P < 0.002$ vs. CON), IRS-2 associated PI-3K (0.5 nM: $610 \pm 23\%$, 5 nM: $318 \pm 22\%$, 25 nM: $290 \pm 23\%$; $P < 0.002$ vs. CON) and its activity (0.5 nM leptin: $450 \pm 49\%$, 5 nM leptin: $348 \pm 27\%$, 25 nM leptin: $215 \pm 18\%$; $P < 0.002$ vs. CON). **Conclusions:** At physiologic concentrations, leptin decreases glucose production by inhibition of the gluconeogenic enzyme PEPCK, which results from stimulation of IRS-2 tyrosine phosphorylation.

OP 42 MODY

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A GENOME SCAN REVEALS HETEROGENEITY AMONG EUROPEAN FAMILIES WITH MATURITY ONSET DIABETES OF THE YOUNG

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Background and Aims: Maturity onset diabetes of the young (MODY) is a single gene disorder characterised by a young onset and an autosomal dominant family history. MODY is heterogeneous, caused by mutations in the glucokinase, Hepatocyte Nuclear Factor (HNF)-1alpha, HNF-4alpha, HNF-1beta and insulin promoter factor 1 genes. We identified 10 MODY families (7 UK, 1 Dutch, 1 Caribbean, 1 Swedish) without mutations in any of the known genes and with a minimum of one member diagnosed < 25 and 2 affected generations. The aim of our study was to identify novel MODY loci to facilitate the identification of further type 2 diabetes genes.

Materials and Methods: We performed a genome wide scan on the 10 'MODYx' families using a MegaBACE 1000 capillary array electrophoresis instrument and 404 microsatellite markers with an average spacing of 10 cM. Linkage analysis was performed using GENEHUNTER 2.0 software using a strict parametric inheritance model with heterogeneity and age related penetrances. A further 20 MODYx families from other European centres were used for fine mapping of candidate regions.

Results: We identified three loci with Heterogeneity Logarithm of the odds (HLOD) scores > 1.0 on chromosomes 3 (HLOD 2.0 - D3S1292 - 149 cM), 16 (HLOD 2.8 - D16S3091 - 109 cM) and 20 (HLOD 2.1 - D20S115 - 21 cM) but none reaching the required significance level of 3.3. Diabetes in two families showed complete co-segregation with markers at the chromosome 3 locus. However, mapping of a 50cM section spanning the chrom. 3 candidate region using 15 additional markers and 20 additional MODYx families did not provide any further evidence for linkage.

Conclusions: The identification of 3 putative MODY loci indicates that further heterogeneity exists among MODYx families. The analysis of additional MODYx families will confirm or refute these loci as regions containing novel MODY genes.

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The response to an oral glucose load alters with genetic aetiology in Maturity-Onset Diabetes of the Young

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Background and Aims: Maturity-onset diabetes of the young (MODY) results from mutations in at least 5 genes. We hypothesized that in patients with MODY the response to an oral glucose load varied with the genetic aetiology and age.

Materials and Methods: Oral glucose tolerance (OGTT) results were collected from 362 subjects from European MODY families who had either a glucokinase (GCK) (n=245) or a hepatic nuclear factor-1 alpha (HNF1a) (n=117) mutation and were not on treatment. **Results:** The two genetically defined groups were of similar BMI ($21.2 \text{ v } 22.0 \text{ kg/m}^2$ $p > 0.05$) and age ($26.7 \text{ v } 26.7 \text{ yr}$ $p > 0.05$). There were clear differences in the pattern of glycaemia. Fasting plasma glucose (FPG) was $> 5.5 \text{ mmol/l}$ in 98% GCK subjects and 54% HNF1a subjects ($p < 0.0001$). GCK subjects had a higher fasting plasma glucose (FPG) (mean (SD): $6.8 (0.8) \text{ v } 6.0 (1.9) \text{ mmol/l}$, $p < 0.0001$), but lower 2 hour values ($8.9 (2.3) \text{ v } 11.2 (5.2) \text{ mmol/l}$, $p < 0.0001$). The relative proportions classified as diabetic depended on whether fasting (GCK 38% v HNF1a 22%) or 2 hour values (19% v 44%) were used ($p < 0.0001$). Fasting hyperglycaemia was present in GCK but not HNF1a patients tested < 10 yr ($6.5 (0.7) \text{ v } 4.6 (0.8) \text{ mmol/l}$ $p < 0.0001$). A significant deterioration in FPG with age occurs in GCK ($r = 0.36$, $p = 0.01$) and HNF1a ($r = 0.34$, $p = 0.01$); this accounts for a deterioration of 0.8 mmol/l in GCK and 3.3 mmol/l in HNF1a over a 70 year life span. However, at 2 hours, although no deterioration in plasma glucose is seen in subjects with GCK ($r = 0.03$, $p > 0.05$), there is a deterioration in 2 hour plasma glucose with age in subjects with HNF1a ($r = 0.32$, $p = 0.01$). **Conclusions:** GCK patients have a glucose sensing beta-cell disorder that raises the fasting glucose from birth with a small increment in response to a glucose load. In HNF1a patients the progressive beta-cell disorder results in a normal fasting glucose in early childhood and higher post glucose load values than GCK that deteriorate with age. In MODY therefore the molecular genetic diagnosis results in clear, age modified, differences in the fasting glucose and the response to an oral glucose load.

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CHARACTERIZATION OF NATURALLY OCCURRING HEPATOCYTE NUCLEAR FACTOR-1 α (HNF-1 α) GENE MUTATIONS.

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Background and Aims: Mutations in the hepatocyte nuclear factor-1 α (HNF-1 α) gene cause MODY3 and more than 80 different HNF-1 α mutations have been identified so far, only a few of which have been functionally studied. Our aim was to study functional effects of five naturally occurring mutations/sequence variations (A98V, L107I, R272C, P291fsinsC and M626K), located in different regions in HNF-1 α gene. **Materials and Methods:** The HNF-1 α mutations (A98V, L107I, R272C, P291fsinsC and M626K) were generated by *in vitro* mutagenesis and subcloned into a pcDNA3.1 expression vector. The transactivation activity of the mutated HNF-1 α proteins was tested using the Dual luciferase assay, with glucose transporter 2 (GLUT2) as the reporter gene. Western blot was performed and DNA binding was studied by electromobility shift assay (EMSA). **Results:** Transactivation activities of the A98V, L107I, R272C, P291fsinsC and M626K were 100%, 21%, 7%, 7% and 46% of that of the wild-type (wt) in HeLa-cells (lacking endogenous HNF-1 α), and 181%, 100%, 19%, 0% and 100% of that of the wt in the MIN6-cells (expressing endogenous HNF-1 α). However, equalising levels of protein expression of M626K to wt in HeLa-cells resulted in similar transcriptional activity. Dominant negative effect was excluded by adding increasing amount of wt in the transfections with L107I. The L107I mutation showed decreased DNA binding, whereas the M626K resulted in increased binding. Western blot performed on the same nuclear extracts demonstrated decreased protein level of L107I and similar protein level of M626K, compared to wt. **Conclusions:** All of the mutated proteins influence transcriptional activity of the HNF-1 α protein. The M626K mutation increases the DNA binding ability, and our results indicate that the L107I mutation is associated with defective translocation from the cytoplasm to the nucleus. These results may help to explain the impaired β -cell function, characteristic of MODY3.

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The mutation phenotype reveals a critical and differing role of the Hepatic Nuclear Factor 1 α and Hepatic Nuclear Factor 1 β in embryological development
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Background and Aims: Maturity-onset diabetes of the young is caused by mutations in the genes encoding the transcription factors HNF1 α (MODY3) and HNF1 β gene (MODY5). HNF1 α and HNF1 β both bind to the same promoter binding sequences and may form hetero- as well as homo-dimers. They are expressed in similar tissues, however in mice HNF1 β is expressed early in embryonic development, whereas HNF1 α is not expressed until during organogenesis. We aimed to compare the phenotypes of patients with HNF1 α and HNF1 β gene mutations to define the differing roles of these two similar transcription factors in man, particularly in embryogenesis. **Materials and Methods:** 20 HNF1 β and 208 HNF1 α mutation carriers were studied. Fasting samples were compared in BMI matched groups: HNF1 β (5), HNF1 α (25) and unaffected family member controls (35). Mutation groups were matched for fasting plasma glucose. **Results:** Non-diabetic renal disease, particularly renal cystic disease is seen in all 20 HNF1 β mutation carriers, but was only seen in 2 HNF1 α mutation carriers ($p < 0.0001$). The histological classification of the renal disease is variable including cystic dysplasia, and hypoplastic glomerulocystic kidney disease. Renal function in the HNF1 β group varied from mild impairment to end stage renal disease (in 1 subject). In 2 families HNF1 β mutations were associated with genital and uterine anomalies. Beta cell function (HOMA %B) was reduced in the HNF1 α subjects (26 (2-37) median (IQ range), $p < 0.001$) and in the HNF1 β subjects (37 (28-70), $p = 0.01$) compared with controls (105 (85-123)). Insulin sensitivity (HOMA%S) was similar in the HNF1 α group (113.2 (60-148)) and control group (99 (66-152)), but was decreased in the HNF1 β group (48 (33-56), $p = 0.01$). Serum Urate was increased in the HNF1 β group (416 $\mu\text{mol/l}$ (89) mean (SD), $p = 0.008$) compared with controls (299 $\mu\text{mol/l}$ (50)) and the HNF1 α group (273 $\mu\text{mol/l}$ (63)). **Conclusions:** Mutations in HNF1 β are specifically associated with non-diabetic renal disease establishing the critical role of HNF1 β in renal development. In both transcription factor diabetes there is beta-cell dysfunction, but in HNF1 β mutations in contrast to HNF1 α mutations, this is associated with insulin resistance and hyperuricaemia. We conclude that the developmental role of the hepatic transcription factors is critical in humans and disruption of this by heterozygous mutations results in discrete, non-pancreatic, clinical features.

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A Mutation of a Novel Splice Variant of HNF-4 α Co-segregates with Maturity-Onset Diabetes of the Young (MODY)

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Background and Aims: Mutations in the transcription factors Hepatic Nuclear Factor (HNF-1 α , HNF-4 α , HNF-1 β and Insulin Promoter Factor-1) genes cause the majority of MODY. The mechanism by which they produce beta cell function and their inter-relationship is uncertain. A splice variant of HNF-4 α utilising a distant upstream promoter and an alternative exon 1 was isolated in mice. Recently this splice variant of HNF-4 α (HNF-4 α 7) was shown to be the predominant HNF-4 α species in the beta cell. In man the alternative promoter has binding sites for the other MODY related transcription factors. This suggests that in contrast to the situation in the liver where HNF-1 α activity is regulated by HNF-4 α , in the beta cell HNF-1 α , HNF-1 β and IPF-1 may regulate HNF4 α through the alternative promoter P2 in the pancreas. The role of genetic variation in the P2 alternative promoter in MODY and early onset type 2 diabetes subjects has not been studied. **Materials and Methods:** We studied 7 European MODYx probands, ie those without mutations in the known genes, and with a minimum of one family member diagnosed < 25 and 2 affected generations, and 32 Early Onset Diabetic subjects (median age at diagnosis 38 (33.5-43), median BMI 30.7 (28.7-37.6)). 452bp of HNF-4 α 7 containing the IPF-1 and HNF-1 α binding sites, in the alternative promoter region and the alternative exon1 was amplified by PCR and sequenced using an ABI 377 DNA sequencer (Applied Biosystems). **Results:** We identified a heterozygous C to T nucleotide substitution in the IPF1 binding site at position -146 in 1 of the 7 MODYx probands. Further examination of 15 family members revealed complete co-segregation of the variant with diabetes with a significant LOD score of 3.25. We did not find any variation in HNF-4 α 7 in the 32 Early Onset Diabetic probands. **Conclusions:** The identification of a MODY family with a cosegregating mutation in the alternative HNF-4 α promoter region establishes a critical role in the human beta cell of this splice variant. It supports the existence of a different transcription factor framework in the beta cell compared to the hepatocytes and suggest previous studies of the role of HNF-4 α in MODY and Type 2 diabetes will need to be repeated in order to examine this region.

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Clinical Aspects of Hypoglycaemia

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A PROSPECTIVE STUDY ON THE PREVALENCE OF SEVERE HYPOLYCAEMIA IN TYPE 1 DIABETIC PATIENTS 1984-1998.

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Background and Aims: A main obstacle to achieve near normal blood-glucose levels in Type-1 diabetes is the risk of hypoglycaemia. Studies performed in the 1980s on large cohorts of adult patients documented prevalences of severe hypoglycaemia between 8-29% and found long duration of diabetes and unawareness of hypoglycaemia to be risk factors for such events. Multiple-injection therapy, self monitoring of blood glucose and new insulin analogs have since been introduced in the routine treatment of these patients.

Materials and Methods: Aiming to study the issue of hypoglycaemia we performed a long term study of a cohort of type 1 diabetic patients registered at our out-patient clinic between 1984 and 1998. An identical questionnaire regarding the previous year was sent to the patients in the beginning of 1985 and 1999 respectively. A subanalysis of patients (n=178) answering both times, is presented here.

Results: The use of multiple injections had increased from 71 to 98% ($p<0.001$) and daily self monitoring of blood-glucose from 17 to 48% ($p<0.001$). An increasing number of patients reported unawareness of hypoglycaemia (54 vs. 40%; $p<0.01$) while no differences were recorded with respect to patient reported anxiety and fear for hypoglycaemia. The prevalence of severe hypoglycaemia had increased from 17 to 27% ($p<0.05$) and nocturnal events were also more frequent (83 vs. 76%; $p<0.05$) in this cohort of patients. In parallel, a slight decrease of HbA1c (7.4 vs. 7.6%; $p<0.05$) was registered.

Conclusions: In spite of more frequent use of multiple injection therapy, use of novel insulin analogs and more frequent self monitoring of blood glucose the prevalence of unawareness of hypoglycaemia increases by time and so does the prevalence of severe hypoglycaemia which remains a major obstacle to achieve good metabolic control.

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INCIDENCE, CLINICAL CHARACTERISTICS AND COSTS OF SEVERE HYPOLYCAEMIA - A PROSPECTIVE POPULATION-BASED STUDY

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Aim: To establish the incidence, clinical characteristics and costs of severe hypoglycaemia (SH) in a German region with 200,000 inhabitants by means of a prospective population-based study with sensitive screening for hypoglycaemia. **Methods:** Severe hypoglycaemia was defined as hypoglycaemia with impairment of consciousness and requiring intravenous glucose or glucagon injection. In order to also detect atypical manifestations of SH, between 1997 and 2000 all 30,768 patients presenting to the medical emergency department of the region's central hospital and 6,631 (85%) of all 7,675 patients attended by the emergency medical service in the region were given an initial blood glucose test. Extensive clinical data were recorded for the patients with hypoglycaemia. **Results:** Altogether 264 cases of SH (blood glucose 39 ± 24 mg/dl) were registered, comprising 14 (5%) cases of spontaneous hypoglycaemia, 179 (68%) cases of SH on insulin therapy, 45 (17%) cases of SH on sulphonylurea therapy and 26 (10%) on combined therapy. There were 91 (35%) cases of SH in type 1-, 145 (55%) in type 2- and 28 (10%) in non-classified diabetic patients. The subgroup analysis confirmed the known risk factors for SH, that is age, comorbidity and polypharmacy in type 2 diabetic patients and recurrent hypoglycaemia and renal failure in type 1 diabetic patients. The acute mortality of the SH was 0.4% (1/264). The overall incidence of SH was 33 per 100,000 inhabitants per year, the incidence of SH on insulin therapy 23 and that of sulphonylurea-induced hypoglycaemia 5.6. The 264 cases of SH led to 1439 days in hospital and caused total costs of 440,000 euros (55,000 euros per 100,000 inhabitants per year; 1,660 euros per case of SH). Due to the longer time spent in hospital (9.5 ± 10.6 vs. 2.3 ± 5.3 days; $p<0.000$) by the older multimorbid patients, SH in type 2 diabetes was considerably more cost-intensive than that in type 1 diabetes. **Conclusions:** SH is a common, cost-intensive complication of diabetes; insulin-induced forms account for the largest number of cases. Hypoglycaemia continues to place high demands on patient education and management in the individual diabetic patient in order to avoid this life-threatening complication and the tremendous costs. The rigorous screening for hypoglycaemia in this prospective study identified a higher incidence of hypoglycaemia than previous retrospective analyses.

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FREQUENCY, SEVERITY, AND MORBIDITY OF HYPOLYCAEMIA OCCURRING AT WORK IN PEOPLE WITH INSULIN-TREATED DIABETES

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Background and aims: Hypoglycaemia is common with insulin therapy and can disrupt everyday activities. Little information is available on hypoglycaemia occurring at work in the insulin-treated population. The frequency and consequences of hypoglycaemia occurring at work were therefore examined in an insulin-treated cohort in regular employment to ascertain the extent and nature of this problem. **Materials and methods:** A prospective 12 month survey of 243 people with insulin-treated diabetes in employment (age range 20-69 years) was performed to record the frequency, severity, and morbidity of hypoglycaemia, with particular emphasis on episodes occurring at work. Details of hypoglycaemic episodes included time of day, place, activity, possible cause, blood glucose, treatment, physical injury, and any other adverse event. Glycaemic control was monitored using serial HbA1c measurements. **Results:** 1951 mild (self-treated) and 242 severe (external help required) hypoglycaemic episodes were recorded. Of the severe hypos, 149 (62%) occurred at home, 36 (15%) at work, and 56 (23%) elsewhere. Half of the severe hypos occurred during sleep. Adverse events were reported in 213 severe episodes, 29 (14%) were comatose, 20 (9%) had a seizure, 4 (2%) sustained a head injury, 5 (2%) another injury, 3 (1%) injured someone else, and 2 (1%) damaged property. No reported road traffic accidents occurred with severe hypoglycaemia but one with mild hypoglycaemia. 187 (78%) of individuals suffering from severe hypoglycaemia reported being treated by a relative or their partner, 31 (13%) reported receiving assistance from a work colleague, and 11 (5%) reported requiring medical help. **Conclusions:** Severe hypoglycaemia is uncommon in the workplace. When it does occur at work, it does not cause disruption. Serious morbidity, including accidents or injuries associated with hypoglycaemia at work, was very uncommon.

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The effects of blood glucose levels on the quality of sleep in children with type I diabetes mellitus

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Background and Aims: Sleep and nocturnal glucose levels in children with type I diabetes may influence each other. Hypo and hyperglycemia can cause sleep disturbances, resulting in cognitive impairment, behavior problems, and growth retardation. Sleep may alter the sensitivity of the arousal system or the counter regulatory hormone response to hypoglycemia, resulting in prolonged nocturnal hypoglycemia.

Materials and Methods: Nine children with type I diabetes mellitus and 8 healthy controls were studied. All underwent full night polysomnographic recordings (EOG, EMG, EEG, ECG, airflow, respiratory effort, O2Sat). Blood glucose levels were measured every 5 minutes in the diabetic children automatically using the MiniMed Continuous Glucose Monitoring System. Frequent glucose level measurements were performed via a subdermal glucose sensor. Glucose levels were measured throughout the night and stored (the values were invisible during the night). The recordings of sleep and glucose levels were analyzed blindly, and then matched and correlated.

Results: 1. All children went to sleep with well-controlled glucose levels (100-200mg%). Four children demonstrated profound hypoglycemia during the night (below 40mg%). Three additional children demonstrated an initial increase followed by a rapid decline in glucose levels. The remaining children had stable glucose levels. 2. The rate of the decrement in blood glucose levels, but not the absolute glucose levels, were associated with awakenings from sleep and sleep disruption. A fall of more than 100mg% glucose per hour was associated with an arousal response. 3. Paradoxically, when hypoglycemia occurred insidiously, the percentage of slow wave sleep increased dramatically, and the EEG recordings at that time demonstrated high amplitude hypersynchronized delta activity. 4. Diabetic children with well-controlled glucose levels had similar sleep pattern as normal age-matched control children.

Conclusions: Rapid changes in glucose levels may disrupt sleep. On the other hand stable hypoglycemia, even if profound, may not result in awakening from sleep and thus may pose a serious risk for complications. Thus, measuring frequent glucose levels during the night adds important data in treating these children.

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High serum ACE activity predicts risk of severe hypoglycaemia in type 1 diabetes

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Background and Aims: We have previously, based on retrospective data, shown that patients with high serum ACE activity are more susceptible to severe hypoglycaemia (SH). In this study we investigated whether our finding could be reproduced prospectively.

Materials and Methods: 170 unselected patients with type 1 diabetes, untreated with ACE inhibitors or angiotensin II receptor antagonists, characterised by C-peptide, level of hypoglycaemic awareness, HbA1c, and clinical data were included in the study. Serum ACE was determined by a commercial assay and ACE genotype by PCR. All patients were followed for one year by monthly questionnaires and reporting of each episode of SH (defined as episodes needing help from other persons) by telephone. Rates of severe hypoglycaemia are reported as episodes per patient-year.

Results: The rates of SH according to the quartiles of serum ACE were 0.8, 0.5, 0.7, and 2.2 in the total material ($p < 0.0001$) and 0.5, 0.8, 0.9, and 3.5 ($p < 0.0001$) in C-peptide negative patients ($n=70$), respectively. The rates of SH according to ACE genotype were: II: 0.8; ID: 1.1; and DD: 1.1 in the total material ($p=0.6$) and: II: 0.3; ID: 1.8; and DD: 1.7 ($p < 0.001$) in C-peptide negative patients. For comparison, the overall rates of SH in patients with or without detectable C-peptide levels were 0.8 and 1.4 ($p < 0.05$) and in patients with normal or impaired awareness the rates were 0.2 and 1.6 ($p < 0.0001$).

Conclusions: Serum ACE is a strong independent predictor of the risk of severe hypoglycaemia in patients with type 1 diabetes, in particular in those with undetectable C-peptide.

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RENAL COMPENSATION FOR IMPAIRED HEPATIC GLUCOSE RELEASE DURING HYPOGLYCEMIA IN TYPE 2 DIABETES

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Background: In overnight-fasted humans both liver and kidney release glucose into the circulation. Under certain conditions (e.g. liver transplantation and prolonged fasting), the kidney increases its release of glucose to compensate for reduced hepatic glucose release. We tested the hypothesis that during counterregulation of hypoglycemia, there would be increased renal glucose release in patients with type 2 diabetes mellitus (T2DM) since they would be expected to have reduced hepatic glucose release because of reduced glucagon responses. **Methods:** Hypoglycemic hyperinsulinemic clamp experiments (~ 3.1 mM) were performed in 12 subjects with T2DM and in 10 age-weight matched nondiabetic volunteers (NV). During these clamps, we measured total endogenous glucose release (TEGR) and renal glucose release (RGR) using a combined isotopic net balance approach as well as counterregulatory hormone responses. We calculated hepatic glucose release (HGR) as TEGR minus RGR since liver and kidney are the only organs able to release glucose. **Results:** Despite comparable hypoglycemia and plasma insulin concentrations, TEGR was reduced in T2DM subjects (6.6 ± 0.6 vs 10.2 ± 1.1 $\mu\text{mol kg}^{-1} \text{min}^{-1}$ in NV, $p=0.01$). This was wholly accounted for by a reduction in HGR (3.9 ± 0.5 vs 8.6 ± 1.0 $\mu\text{mol kg}^{-1} \text{min}^{-1}$ in NV, $p=0.0015$) since RGR was increased in T2DM subjects (3.3 ± 0.5 vs 1.6 ± 0.3 $\mu\text{mol kg}^{-1} \text{min}^{-1}$ in NV, $p=0.015$). During hypoglycemia plasma epinephrine, lactate and free fatty acid concentrations were greater in the T2DM subjects (all $p < 0.01$) and net renal lactate uptake was significantly correlated with RGR ($r=0.6$, $p=0.01$). **Conclusions:** During counterregulation of hypoglycemia, increased renal glucose release, probably due to increased epinephrine responses, partially compensates for reduced hepatic glucose release in patients with T2DM. These observations may explain why patients with T2DM have a relatively low occurrence of severe hypoglycemia compared to patients with type 1 diabetes (T1DM). Loss of this compensatory increase in renal glucose release in patients with type 1 diabetes mellitus who have deficient epinephrine counterregulatory responses and in patients with T2DM who develop endstage renal disease may explain why these patients are prone to severe hypoglycemia.

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PREVIOUS HISTORY OF HYPOGLYCEMIC COMA DETERMINES COGNITIVE AND EEG ABNORMALITIES DURING HYPOGLYCEMIA IN TYPE 1 DIABETIC PATIENTS.

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Aims: Type 1 diabetic patients with recurrent severe hypoglycemia are at increased risk for subsequent hypoglycemic comas. It is still not clear, however, whether patient's previous exposure to hypoglycemic coma could lead to permanent cognitive dysfunction. The aim of our study was to determine whether previous history of hypoglycemic coma determines early cognitive dysfunction and EEG abnormalities during controlled hypoglycemia in type 1 diabetic patients. **Methods:** 6 type 1 diabetic patients (5 M and 1 F) (mean age 37 ± 4 yrs, HbA1c $8.1 \pm 1\%$, duration 17 ± 4 yrs) with at least 2 hypoglycemic comas in the previous 3 years (SH) and 6 matched type 1 diabetic patients with no history of severe hypoglycemia (NH) were studied with a slow-fall hypoglycemic clamp (insulin infusion $= 1.5$ mU/kg/min, plasma glucose reduced stepwise to 68, 58 and 49 mg/dl every 40 minutes). Cognitive function (4-Reaction time), symptomatic responses (semi quantitative questionnaire) and EEG spectral analysis activity were recorded throughout. **Results:** During hypoglycemia cognitive function deteriorated significantly (4-RT) at a plasma glucose threshold of 55 ± 1 mg/dl in both groups ($p=ns$). However, in SH autonomic symptoms were significantly diminished (plasma glucose threshold 49 ± 1 vs. 54 ± 1 mg/dl, $p=0.02$). During cognitive task in SH patients EEG spectral analysis demonstrated 1) a slowing in beta activity at euglycemia (24 ± 1 vs. 14 ± 1 Hz, $p < 0.03$); 2) a slowing of theta activity (13 ± 1 vs. 22 ± 2 Hz, $p < 0.05$), beta activity, (25 ± 1 vs. 14 ± 1 Hz, $p < 0.05$) and absolute power (6 ± 1 vs. 11 ± 2 Hz, $p=0.07$) during controlled hypoglycemia (plasma glucose 56 ± 1 mg/dl). Between the two groups, a significant interaction effect was found between plasma glucose and theta activity (ANOVA; $F=7.4$, $p < 0.05$). Moreover, during eye closed EEG recording only in SH patients a high significant correlation was found between glucose levels and EEG spectral analysis (beta: $r=0.52$, $p < 0.001$, theta: $r=0.59$, $p < 0.001$, MDF: $r=0.62$, $p < 0.001$). **Conclusions:** Controlled hypoglycemia elicits cognitive dysfunction in both NH and SH patients. However only patients with previous history of hypoglycemic coma present significant EEG alterations during hypoglycemia. This suggests that hypoglycemic coma determines a specific susceptibility to the metabolic encephalopathy.

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A Novel Treatment of Hypoglycemia Unawareness (HU) with Anti-Diabetic Sulfonylureas Taro Wasada and Yasuhiko Iwamoto, Tokyo, Japan

Background and Aims: The most serious aspect of HU is that neuroglycopenia precedes the onset of counter-regulation and autonomic nerve activation, thereby leading to cognitive dysfunction, such as loss of personal control, abnormal behavior, and even unconsciousness and seizure. So far, the only strategy for prevention of HU is the meticulous avoidance of hypoglycemia (H), which is hardly achieved. Here we propose a potentially effective treatment for this life-threatening problem in patients at risk. **Case 1:** a 43 y/o m. type 1 diabetic with 9 ys of duration. Ten mo ago, his abnormal behavior was found during sleep; he moved his arms involuntarily, and occasionally shouted. Neurological examinations including brain MRI, EEG and brain blood flow study were all normal. Based on his blood glucose monitoring at home (SMBG), nocturnal H was evident. When the potentially beneficial effect of SU drugs was explained, he and his wife expressed a strong desire to receive SU. During a 4 w-Tx with glimepiride (GLP) 2mg at bedtime, abnormal behavior ceased. He was instructed to take GLP 2mg a day for 2 w and stop it for additional 2 w. While on GLP, previous episodes were not observed, and occasionally he felt "light-headedness" at blood glucose levels below 2.2mM. While off GLP, he became less alert on two occasions, and couldn't detect H at blood glucose levels of 1.4 to 2.7mM. Abnormal behavior, which occurred previously 2 to 4 times per mo, didn't recur on the Tx with GLP 4mg/day for 4 mo, but its discontinuation again caused the symptoms at night. His perception of H remains compromised. **Case 2:** a 52 y/o m. insulin-treated diabetics. Six mo ago, he suddenly lost consciousness. Thereafter, he experienced several episodes of unconsciousness with tonic clonic seizures. HU was evident from his SMBG. With strong request by the individual and his medical supervisor, 1 mg of GLP twice a day was begun. Since then he has had no episodes of unconsciousness over the last 6 mo. He sometimes experienced "light-headedness" and slight sweating at blood glucose levels around 2.8mM, but didn't notice H on the majority of occasions. **Case 3:** a 53 y/o f. type 1 diabetic with 10 ys of duration. Her average HbA1c level of the recent 12 mo was 6.5%. She experienced nocturnal seizures three-times during the last two years. She began to receive 2mg GLP at bedtime. Since then she began to feel "something is wrong" when blood glucose fell below 2.5mM. Based on her SMBG, she was able to recognize H in approximately 80% of 13 documented episodes of H (< 3.3 mM) during GLP Tx, while 50% of 16 documented episodes of H during its withdrawal. **Conclusions:** The glucose-receptive neurons in specific regions of the brain sense ambient glucose concentrations by their ATP-sensitive potassium (KATP) channels and thereby contribute to counter-regulation, autonomic nerve stimulation, seizure protection, and integrity of cognitive function. Therefore, it is tempting to speculate that KATP channels of these neurons are defective in HU. Our preliminary findings favor an idea that SU might enhance cognitive function by affecting neuronal excitability and neurotransmitter release through antagonizing H-induced activation of KATP channels.

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Treatment of Diabetes and Myocardial Function

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BENEFICIAL EFFECTS OF C-PEPTIDE ON MYOCARDIAL FUNCTION IN PATIENTS WITH TYPE 1 DIABETES

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Background and Aims. Proinsulin C-peptide (CP) has recently been shown to bind specifically to a G-protein coupled membrane receptor and to stimulate Na^+/K^+ -ATPase and eNOS activities via Ca^{2+} -dependent signaling pathways. CP increases blood flow in skeletal muscle, probably by recruitment of capillaries, when administered to patients with type 1 diabetes (D). Myocardial effects of CP are examined in this study.

Methods and Materials. Eight male D (mean age 28 ± 5 yrs) without signs of cardiovascular disease during normoglycemia were studied on two separate occasions using a double blind study design: one day with infusion of CP (5 pmol/kg/min) during 60 min, and on another day with saline infusion. Eight volunteers (N) served as age matched controls. Peak regional myocardial velocities during early diastole (Vd) and systole (Vs) were measured using pulsed tissue Doppler at rest and after diprydamole stress (DS, 0.84 mg/kg) both before and after CP. Myocardial contrast echo was performed using pulse inversion technique. The plateau signal intensity (A), which correlates with the myocardial blood volume, was calculated from digital signal intensity versus pulsing interval plots.

Results. Vd at rest was reduced in D ($13.8 \pm 1.6 \text{ cm/s}$) compared to N ($15.5 \pm 2.7 \text{ cm/s}$, $p < 0.01$) and so was Vs at rest (8.0 ± 1.0 vs $8.8 \pm 1.6 \text{ cm/s}$, $p < 0.02$). During CP infusion, Vd and Vs increased by $12 \pm 3\%$ ($p < 0.01$, from 13.8 ± 1.6 to $15.4 \pm 1.7 \text{ cm/s}$) and by $12 \pm 4\%$ ($p < 0.05$, from 8.0 ± 1.0 to $8.9 \pm 1.5 \text{ cm/s}$), respectively. During DS, Vd increased from 13.8 ± 1.6 to $15.5 \pm 1.4 \text{ cm/s}$ and Vs from 8.0 ± 1.0 to $9.9 \pm 1.1 \text{ cm/s}$. A at rest increased from $6.5 \pm 3.2 \text{ dB}$ to $8.3 \pm 3.3 \text{ dB}$ during CP ($p < 0.01$) and to $11.2 \pm 5.2 \text{ dB}$ during DS ($p < 0.001$) in D. No significant changes were observed during saline infusion. These data indicate that C-peptide may exert beneficial effects on systolic and diastolic myocardial contraction velocities and perfusion in type 1 diabetic patients.

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THE EFFECT OF INTENSIVE INSULIN THERAPY ON QT DISPERSION DURING ACUTE CORONARY EVENTS IN DIABETIC PATIENTS.

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Background and Aims: The link between the dispersion of ventricular recovery times, myocardial arrhythmogenicity and increased mortality has been extensively studied. Aim of our study was to evaluate the hypothesis that strict metabolic control with insulin infusion in type 2 diabetic patients (pts) with non-ST segment elevation Acute Coronary Syndromes (non-ST ACS), may influence QT dispersion (QT disp). **Materials and Methods:** The study included 48 type 2 diabetic pts with non-ST ACS, 23 randomized to conventional treatment plus intensive insulin therapy (Group A) and 25 to conventional therapy only (Group B). Group A pts received insulin by infusion for 72 hours, aiming to maintain normoglycaemia. Group B pts were treated according to Coronary Care Unit standard practice. The maximum QT (QT max), the minimum QT (QT min) and QT disp (QT max-QT min) were determined on two occasions: analyzing the first near normal ECG at a time point as soon as possible after admission and 72 hours later. Also, a Doppler index (DI), designed to determine the global myocardial performance, defined as the sum of isovolumetric contraction plus isovolumetric relaxation time divided by ejection time, was estimated in two serial echo studies, on admission and 72 hours later. The two groups were comparable in terms of medical history, clinical, echocardiographic and biochemical data. Pts with, a) a previous myocardial infarction, b) evolution in ST elevation AMI (ST-AMI), c) left ventricular (LV) hypertrophy and, d) intraventricular conduction disturbances, were not recruited. **Results:** Two pts from both groups were excluded from the analysis because there was evidence for evolution in ST-AMI. Mean Glucose level in the infusion group was significantly lower than in controls (6.98 mmol/l^1 vs. 11.19 mmol/l^1 , $p < 0.001$). QTmax, QTmin and QTdisp were similar on admission in the two groups (Group A vs. Group B; QTmax: 413 ± 22 vs. $409 \pm 25 \text{ msec}$; QTmin: 356 ± 24 vs. $349 \pm 30 \text{ msec}$; QTdisp: 57 ± 28 vs. $60 \pm 23 \text{ msec}$, $p \text{ NS}$). At 72 hours, Group A patients showed significantly improved DI and QTdisp values (Group A vs. Group B; DI: 0.64 ± 0.17 vs. 0.73 ± 0.11 , $p < 0.05$; QTdisp: 49 ± 21 vs. $62 \pm 16 \text{ msec}$, $p < 0.01$) when compared with Group B pts. However, the multivariate analysis revealed that type of treatment did not independently influence the QTdisp values, due to a strong dependency of QTdisp values on improvement of LV function (Beta = 0.56 , $p < 0.001$). **Conclusion:** Intensive insulin treatment during non-ST ACS improves QTdisp, although this phenomenon could be attributed to an enhancement of LV function.

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Diastolic Myocardial Function and Myocardial Microvasculature Reserve Improve with Intense Insulin Treatment in Type 2 Diabetic Patients.

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Background and aims: Diastolic dysfunction and reduced myocardial flow reserve precede systolic abnormalities, coronary artery disease and overt microangiopathy in diabetic patients (D). We tested the hypothesis, that this may be reversed by normalisation of hyperglycemia by means of intense antidiabetic treatment (IAT) in type 2 D. **Methods:** 30 D (mean age 59 ± 8 years) had systolic (Vs) and diastolic (Vd) regional myocardial velocities assessed with pulsed Doppler tissue imaging in 12 left ventricular segments during diprydamole stress (DS) echocardiography (0.84 mg/kg). Simultaneously, myocardial perfusion was assessed by contrast echocardiography after i.v. infusion of Levovist® (4 g) using pulse inversion technique at 1.3 mechanical index. The plateau value (MBV), which correlates with myocardial blood volume, was derived from myocardial signal intensity measurements at increasing trigger intervals and normalized for signal intensity within the LV cavity (%). 23 D were studied before and after 3 weeks of IAT and 7 D during unchanged treatment (C). **Results:** In C, fasting B-Glucose, Vd, Vs and MBV remained unchanged. After IAT, fasting B-Glucose decreased by 3.6 mmol/L , Vd increased from $7.8 \pm 1.5 \text{ cm/s}$ to $8.6 \pm 1.4 \text{ cm/s}$ ($p < 0.002$), Vs remained unchanged (6.2 ± 1 and $6.4 \pm 0.9 \text{ cm/s}$) and MBV during DS was increased from 18 ± 8 to $22 \pm 8\%$ ($p < 0.001$). Vd increased from 7.3 ± 1.4 to $8.3 \pm 1.4 \text{ cm/s}$ ($p < 0.002$) in the subgroup of 13 patients whose improved glycemic control was achieved by increased insulin dose during IAT. Vd remained unchanged in the 10 D in whom a comparable IAT was achieved via oral drugs without modification of insulin. The changes in diastolic function were paralleled by the changes of MBV at maximal vasodilatation in both subgroups. **Conclusion:** Diastolic dysfunction in diabetic patients improves by intensive insulin treatment. This beneficial effect may be due to augmented availability of myocardial microvasculature.

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Interactions between nicorandil and sulphonylureas: a structural basis.

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Background and Aims: Nicorandil is an anti-anginal agent that opens ATP-sensitive potassium (K-ATP) channels in smooth muscle thereby vasodilating the coronary vasculature. K-ATP channels in muscle and pancreatic beta cells share a common pore-forming subunit, Kir6.2, but possess alternative sulphonylurea receptors (SUR1 in beta cells, SUR2A in cardiac muscle and SUR2B in smooth muscle). The aim of this study was to investigate the domains of the K-ATP channel involved in nicorandil activity, and to determine whether the drug interacts with hypoglycaemic sulphonylureas. **Materials and Methods:** We expressed recombinant K-ATP channels in *Xenopus* oocytes and measured the effects of drugs and nucleotides by recording macroscopic currents in excised membrane patches. **Results:** Nicorandil activated Kir6.2/SUR2A and Kir6.2/SUR2B but not Kir6.2/SUR1 currents, consistent with its specificity for cardiac and smooth muscle K-ATP channels. Drug activity was dependent on the presence of nucleotides and was impaired when the Walker A lysine residues were mutated in either nucleotide binding domain of SUR2. To identify domains essential for nicorandil activity, we constructed chimeras between SUR1 and SUR2A. Swapping TMs 13-17 (but not TMs 13-16 alone) from SUR2A to SUR1 transferred nicorandil sensitivity. The reverse chimera, in which TMs 13-16 of SUR1 were transferred into SUR2A, was only partially activated by nicorandil. Thus, both TM17 and TMs 13-16 of SUR2A are required for nicorandil activity. This domain corresponds to the region of SUR1 that contains the sulphonylurea binding site. As sulphonylureas exhibit different specificities for SUR1 and SUR2-types of K-ATP channel, we investigated the interaction between sulphonylureas and nicorandil. In the presence of ATP, 0.1 mM nicorandil activated Kir6.2/SUR2A and Kir6.2/SUR2B currents 4.1 ± 0.8 ($n=20$) and 4.2 ± 1.2 -fold ($n=18$), respectively ($\text{mn} \pm \text{sem}$). The response was unaffected by 0.01 mM gliclazide (SUR2A: 3.1 ± 0.5 ($n=7$); SUR2B: 3.7 ± 1.4 -fold ($n=5$) activation), but was severely impaired by 100 nM glibenclamide (SUR2A: 0.5 ± 0.1 ($n=6$); SUR2B: 0.9 ± 0.1 -fold ($n=6$) activation) or 100 nM glimepiride (SUR2A: 0.6 ± 0.1 ($n=7$); SUR2B: 2.1 ± 0.3 -fold ($n=7$) activation). **Conclusions:** Nicorandil specifically targets SUR2-type K-ATP channels due to its interaction with the C-terminal group of TMs of SUR2. Nucleotides modify the response via the nucleotide binding domains of SUR. Nicorandil activity was unaffected by gliclazide, which specifically blocks SUR1-type K-ATP channels, but was severely impaired by glibenclamide and glimepiride, which target both SUR1 and SUR2-type K-ATP channels.

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Effects of Treatment with Sulfonylurea Drugs or Insulin on Ischaemia Induced Myocardial Dysfunction in Type 2 Diabetes Mellitus.

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Background and Aims: In patients with Type 2 diabetes (DM2) and coronary artery disease (CAD), the potential negative role of sulfonylurea drugs on ischemic preconditioning, is under debate. We assessed the effect of treatment with glibenclamide (G) or I on ventricular myocardial dysfunction (MD) induced by acute ischemia in 19 patients with DM2 and CAD.

Materials and Methods: Each patient (M/F=14/5; age=61±2yrs; BMI= 29.3± 0.8 kg/m²) was randomly assigned to either I or G treatment and crossed-over after 12 weeks for an additional similar period. Metabolic control was maintained constant during both treatments. At the end of each period left ventricular end diastolic volume index (LVEDVI), ejection fraction (LVEF), and wall motion score index (WMSI) were studied by 2D echocardiography at rest and during dipyridamole infusion (DI) (0.84mg/kg over 10min). Ten healthy matched subjects (C) (M/F=8/2; age=60±4yrs; BMI= 28.0±0.8 kg/m²) served as control for baseline echocardiographic evaluation.

Results: LVEDVI (G=109±20; I=109±19 ml/m²), LVEF (G=43±7; I=46±8%), WMSI (G=1.40±0.29; I=1.40±0.28) were similar with G or I at rest, while in DM2, LVEDVI was higher and LVEF was lower compared to C (64±9 ml/m² and 60±9% respectively; both p<0.005), independently of treatment. During DI, after G treatment, peak stress LVEF (37±12 vs 43±7%; p<0.005) was reduced and WMSI (1.98±0.24 vs 1.4±0.28; p<0.001) was increased significantly, while after I treatment, peak stress LVEF (45±11 vs 46±8%) and WMSI (1.60±0.40 vs 1.4±0.29) did not differ from baseline. LVEF was significantly higher and WMSI was significantly lower with I than with G (both p<0.001).

Conclusions: In patients with DM2 and CAD, ischemic MD induced by DI is more severe during treatment with G than with I. Reconstitution of a preconditioning mechanism by I may have a potential beneficial effect.

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Metabolic and Endothelial Effects of Trimetazidine in Type 2 Diabetic Patients with Ischemic Dilated Cardiomyopathy.

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Background and Aims: Aim of the study was to evaluate whether trimetazidine (TMZ), a compound known to inhibit myocardial beta-oxidation, can improve peripheral and skeletal muscle (SM) glucose utilisation, exercise tolerance and resting ventricular function in 16 type 2 diabetic patients affected by severe ischemic dilated cardiomyopathy.

Materials and Methods: All patients were randomly allocated in a double-blind study in which TMZ (40 mg/day) or placebo were administered for 15 days. At the end of each period, they underwent euglycaemic hyperinsulinaemic clamp and forearm indirect calorimetry, 2D-echocardiography and exercise testing.

Results: Compared to placebo, TMZ decreased fasting glucose levels (6.7±0.4 vs 7.6±0.6 mM; p<0.05) increasing SM glucose oxidation (0.68±0.08 vs 0.48±0.06 μM/100 mL/min; p<0.05) without changes in insulin levels. At the end of the clamp, TMZ increased M-value (4.0±0.5 vs 3.3±0.4 mg/kg/min; p<0.01), SM glucose utilisation (4.03±0.61 vs 2.95±0.64 μM/100 mL/min; p<0.03) and SM glucose oxidation (1.84±0.14 vs 0.88±0.16 μM/100 mL/min; p<0.001) while SM lipid oxidation was completely inhibited (p<0.002). No changes in SM glucose storage were observed. A significant increment of ejection fraction (46±6 vs 43±6%; p<0.03), fractional shortening (27±7 vs 24±7; p<0.05) and in maximal exercise time (450±154 vs 426±167 sec; p=0.08) were observed. In parallel, basal endothelin-1 levels significantly decreased after TMZ (11.3±2.6 vs 13.6±2.7 pg/mL; p<0.05).

Conclusions: TMZ improved glucose metabolism and left ventricular function in type 2 diabetic patients with dilated ischemic cardiomyopathy. These effects are likely to be dependent on the fact that TMZ shift FFA oxidation to glucose oxidation and may be operative in both the heart and the vascular endothelium.

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INFLUENCE OF ANTIDIABETIC TREATMENT WITH SULFONYLUREA DRUGS ON LONG-TERM SURVIVAL AFTER ACUTE MYOCARDIAL INFARCTION IN TYPE 2 DIABETIC PATIENTS

The LAngendreer Myocardial infarction and Blood glucose in Diabetic patients Assessment (LAMBDA)

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Introduction: Type 2 diabetic patients show a significantly higher mortality after acute myocardial infarction compared to non-diabetic patients. The influence of sulfonylureas on the survival after acute myocardial infarction is still under debate.

Patients and methods: Survival of 562 patients, consecutively admitted to an intensive care unit with the diagnosis acute myocardial infarction was prospectively assessed for > 3 years. At the time of hospital admission, patients were grouped as (a) non-diabetic patients; (b) newly diagnosed type 2 diabetic patients; (c) patients with known type 2 diabetes not treated with sulfonylureas and (d) patients with known type 2 diabetes treated with sulfonylureas. Survival-analysis was performed according to Kaplan-Meier.

Results: 324 patients were non-diabetics, in 86 cases type 2 diabetes was newly diagnosed at the time of hospital admission, 77 patients with known diabetes had taken sulfonylureas (glibenclamide in all cases) prior to the acute myocardial infarction, 75 patients were on any other antidiabetic treatment. Long-term-survival was significantly shorter in type 2 diabetic patients compared to the non-diabetic patients (p < 0.0001). However, no significant differences were observed between the type 2 diabetic patients treated with sulfonylurea-drugs and those receiving any other antidiabetic treatment (p = 0.60).

Conclusions: An antidiabetic treatment with sulfonylurea-drugs prior to acute myocardial infarction does not have negative effects on the long-term survival. Larger prospective studies will be necessary to finally clarify this question.

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EFFECT OF CARDIAC REHABILITATION AFTER AN ISCHEMIC HEART EVENT ON CARDIOVASCULAR CAPACITIES, IN TYPE 2 DIABETES.

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Background and Aims: cardiac rehabilitation (CR), after an ischemic heart event, significantly improves cardiovascular capacities, in the general population. However, we do not know, so far, whether diabetic patients can obtain from CR a similar benefit than non diabetic subjects. **Methods:** In order to answer this question we performed a controlled prospective study in 38 type 2 diabetic patients (31 M, 7 F), included in a CR program which was started less than one month after an ischemic heart event treated by fibrinolysis and/or angioplasty. The patients had a 2-month CR program, including 60-minute physical exercise sessions 3 times a week. A cycling exercise test including VO₂max measurement was performed in each patient at the beginning then at the end of the CR program. The results obtained in diabetic patients were compared to those from a non-diabetic control group matched for age, sex ratio, left ventricular ejection fraction, ischemic heart event type and treatment. **Results:** Before CR no differences between diabetic and non diabetic patients were observed for maximal power and VO₂max during the exercise test. At the end of the 2-month CR program, diabetic patients showed, during the final exercise test, significantly lower maximal power (118±27 vs 138±35 watt, p=0.01) and VO₂max (23±7 vs 29±8 mL/min/kg, p=0.003). Improvement of cardiovascular capacities was significantly reduced in diabetic patients compared to non-diabetic subjects especially maximal power (18.6±15 vs 29.4±20 watt, p<0.05), heart rate at exercise (5±10 vs 17±15 beat/min, p<0.001) and VO₂max (2.5±4 vs 6.4±5, p=0.001). Among the 38 diabetic patients, we could separate a group of 21 patients who obtained a significant benefit from CR (VO₂ improvement >5%) and a group of 17 patients who had no benefit (VO₂ improvement <5%). In a multivariate analysis, only HbA_{1c} could significantly differentiate these 2 groups (6.6±1 vs 8.3±2 %, p<0.05) when age, lipids, microalbuminuria, neuropathy, leg arteriopathy, left ventricular ejection fraction, ischemic heart event type and treatment were not different between the 2 groups. **Conclusions:** 1) The benefit (power, VO₂ max) of cardiac rehabilitation after an ischemic heart event is reduced in diabetic patients. 2) Poor glycaemic control is the main factor explaining the absence of cardiovascular capacities improvement, after cardiac rehabilitation, in some diabetic patients.

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Beta-Cell Growth and Differentiation

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Mechanisms of Sertoli's cell-induced mitogenic effects on isolated rat islet B-cells: in vitro morphologic and functional, and in vivo post-transplant consequences.

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Background and Aims: The low mitotic activity of adult islet (I) B-cells may impair the long-term I transplant (TX) functional life-span. We had previously observed that co-incubation of rat Sertoli's cells (SC) with homologous I induced significant increase in B-cell mitogenesis. However, the nature of the involved mechanisms remained unclear. We aimed to clarify essence and specificity of the SC-related mitogenic effects, as well as their potential impact on I TX.

Materials and Methods: Adult Spague-Dawley (SD) rat I were incubated with either homologous pre-pubertal SC, or SC concentrated supernatant (S). Control astrocytes (AA) separated from SD rat brain cortex were co-incubated with I. After 12 days, the preparations were incubated with 5-bromodeoxyuridine (BrdU), and double-stained with fluorescein (FITC)-conjugated mouse anti-BrdU monoclonal antibodies (green signal) and tetrahydroamine-isothiocyanate (TRITC)-conjugated mouse anti-insulin antibodies (red signal). The samples were imaged on laser confocal microscopy (LCM). Insulin release from SC-I, SC-S and AA-I, statically incubated with glucose at different concentrations (2.5 through 16.7 mM), was determined. I and I+SC clusters were enveloped in our alginate/poly-L-ornithine microcapsules, and grafted into two groups of CD-1 mice (n=5+5) with streptozotocin-induced diabetes. Blood glucose was monitored throughout the study.

Results: Unlike I alone and I+AA, I+SC and I+S showed very bright fluorescence patterns, reflecting higher insulin signals, under LCM examination. Mitotic nuclei, as identified by BrdU positive staining, coincided with those of b-cells. In particular, quantification of BrdU-positive B-cells showed that the b-cell mitotic rate was significantly higher for I+SC (8.1%) and I+S (7.4%) as compared to I and I+AA (1%), $p < 0.01$. In vitro insulin release was significantly greater from I+SC and I+S than for I and I+AA, upon either basal or high-glucose stimulation ($p < 0.01$). Normoglycemia was restored in all recipients, regardless of the TX setting. However, at 120 days of post-TX, 3/5 mice grafted with encapsulated I+SC, while none of the control encapsulated I-alone group, were still on remission of hyperglycemia.

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R Redifferentiation of expanded human beta cells to insulin producing cells

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Background and Aims: The need for transplantable human islets has stimulated strategies to produce islet neogenesis and/or initiate islet growth. In the healthy adult pancreas, the cell proliferative capacity is low, however, pancreatic cell replication, can be induced under experimental conditions. In vitro studies on beta cell proliferation performed on extracellular matrices plus growth factors have demonstrated a possible cell replication which was however accompanied by a loss of insulin secretion. The aim of this study was to try to reexpress human beta cell secreting capacity after in vitro cell expansion of human beta cells.

Materials and Methods: The core of human pancreatic islets isolated from 5-7 cadaveric donors and consisting mainly in beta cells, were cultured on HTB-9 cell matrices plus 10 ng/ml HGF with the addition or not of compounds known for their stimulatory properties on cell differentiation and/or insulin secretion. The potency of the tested compounds (Activin-A, TGF- β , calcitriol, sodium butyrate, GLP-1, Exendin-4, Nicotinamide and some of their association), was assessed on insulin, PDX-1, Glut1, Glut3 and glucokinase gene expression after 14 day culture as well as on insulin secretion.

Results: In proliferating control cells, the remaining insulin secretion was 10% of starting culture after 14 day culture and the remaining insulin gene expression only 5%. The expression of the other genes implicated in the control of insulin synthesis and secretion was 36%, 66%, 75%, and 87% for PDX-1, glucokinase, Glut1 and Glut 3 respectively compared to starting conditions.

Reversal potencies were mainly observed with sodium butyrate which, compared to control cultures, enhanced 2-3 fold the insulin, PDX-1 and glucokinase gene expression, and the association nicotinamide plus sodium butyrate which produced a 2 fold increase of insulin secretion. In contrast, Glut1 gene expression was up regulated by TGF- β .

Conclusions: Thus, expanding dedifferentiated beta cells can be directed to redifferentiate under stimulating culture conditions. This preliminary approach may be of interest as a modality for treatment of type I diabetes.

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IDENTIFICATION OF TUBULAR COMPLEXES WITH PLURIPOTENT STEM CELLS IN THE PANCREAS OF DIABETES-PRONE BB RATS

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Background and Aims: Tubular complexes (TC) have been reported in pancreatitis, ductal wrapping, chemically induced pancreatic carcinogenesis, pancreatectomy, and streptozotocin-induced diabetes. However, these structures have not been documented in spontaneous models of type 1 diabetes, such as the diabetes-prone BioBreeding (BBdp) rat.

Materials and Methods: Groups of BBdp and BB control (BBc) rats with 10-40 animals were fed a standard cereal-based rodent diet from weaning until 150 d. Pancreata from rats aged 7-150 d were fixed in Bouin's solution. Pancreatic sections were stained with H&E, and antibodies for insulin, glucagon, homeodomain-containing transcription factor pancreas duodenum homeobox gene-1 (PDX-1), keratin, cytokeratin 20 (CK20), PCNA, and nestin.

Results: TC were seen infrequently (10%) in both BBc and BBdp rats younger than 30 d, were absent between 30-52 d and re-appeared only in BBdp and diabetic rats older than 52 d. The frequency of TC increased in BBdp rats to 40% around 90 d, which is similar to the mean age of onset of diabetes. Typical TC were composed of duct-like structures with low cuboidal or flattened cells (keratin positive, CK 20 negative) surrounding a large acinar lumen, and islets with insulin positive, glucagon positive, PDX-1 positive, keratin negative, and CK 20 negative cells. Islets outside of the TC were keratin positive. Non-islet regions of TC were composed mainly of acinar, ductule and inflammatory cells. Duct-like acinar cells were PDX-1 negative and characterized by a loss of zymogen granules, increased expression of keratin, PCNA and increased apoptosis. Increased expression of nestin, a marker of pluripotent cells in the pancreas, was found in TC. TC in animals subjected to pancreatic duct wrapping, a means of enhancing islet neogenesis, were 6 times more frequent than in sham controls.

Conclusions: We showed, for the first time, that TC are normally infrequent in BB rats, but recur in adolescent BBdp rats with pancreatic islet injury by the de-differentiation of acinar cells into pluripotent duct-like cells from which new islets bud. These data suggest that the formation of tubular complexes in the pancreas is a form of neogenesis that is an attempt to compensate for islet injury. (Supported by JDF, CIHR, ORDCF, and Health Canada)

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Effect of sodium tungstate upon pancreatic islet growth.

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Background: Administration of sodium tungstate (Na₂O₄W) to different animal of Diabetes Mellitus (STZ and nSTZ) normalises blood glucose levels.

Aim: To demonstrate the possible effect of Na₂O₄W upon B-cell replication, islet neogenesis and B-cell apoptosis.

Material and Methods: We have used different experimental groups: a) Normal Wistar-Furth rats, b) STZ-Wistar-Furth rats, and c) STZ-treated Wistar-Furth rats transplanted with isogenic islets (STZ+Tx) under the renal capsule (number of transplanted islets insufficient to maintain normoglycemia). All groups have an additional subgroup receiving Na₂O₄W in the drinking water (2 mg/ml). Serum glucose and insulin levels were periodically measured, while immunomorphometric studies were performed in section of fixed pancreas, B cells Vvi, B-cell replication rate (PCNA), islet neogenesis (Cytokeratins), and B-cell apoptosis (Propidium iodide).

Results: Na₂O₄W administration to STZ+Tx diabetic animals normalised glucose values (108.67±5.0 mg/dl vs 494.00±5.0 mg/dl; $p < 0.001$). Changes in glycemia were accompanied by a significant increase in serum insulin levels (3.72±0.99 ng/ml vs. 0.88±0.23 ng/ml; $p < 0.01$). Islet B-cell Vvi: Na₂O₄W did not induce significant changes in control and in STZ animals, but increased 25 times in STZ+Tx rats. Extra islet B-cell Vvi: Na₂O₄W induced an increase of 1.3 times in control rats, of 4 times in STZ + Tx. Acinar PCNA: Na₂O₄W induced an increase of 49 times in control animals, of 8 times in STZ rats, and of 420 times in the STZ+Tx. Ductal PCNA: Na₂O₄W induced an increase of 22 times in control animals, of 9 times in the STZ group, and of 6 times in the STZ+Tx group. Insular PCNA: Na₂O₄W induced an increase of 102 times in the control group, and of > 1000 times in the STZ+Tx group, while no changes were observed in the STZ group. Na₂O₄W induced a marked increase in the size and insulin immunoreactivity of B cells of the islets transplanted under the renal capsule. B-cell apoptotic rate: it was a marked increase in STZ animals, that decreased 14 times after Na₂O₄W administration. This effect was more marked in the STZ+Tx animals. Signs of increased neogenesis (immunocytochemical positive stain) were observed in all groups after Na₂O₄W administration.

Conclusion: the marked stimulatory effect of W upon B-cell replication rate and islet neogenesis, together with its inhibitory effect upon B-cell apoptotic rate, and the consequent amelioration of glucose homeostasis, suggest that it could be a suitable alternative for the treatment of conditions characterized by a decrease in B-cell mass.

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P38 MITOGEN ACTIVATED PROTEIN KINASE (MAPK) SIGNALLING IS REQUIRED FOR HUMAN BETA-CELL DIFFERENTIATION

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Background and Aims: Recently we developed the first human pancreatic pre beta-cell line, Blox5, that is able to differentiate into glucose responsive insulin secreting cells when expressing Pdx-1 during stimulation by the GLP-1 analogue exendin-4 (Ex4) and pseudo-islet formation. While there has been much progress in the understanding of transcription factor expression in beta-cell differentiation, the signalling pathways that determine and maintain the endocrine cell phenotype are poorly understood. Therefore, the aim of this study was to investigate different signalling pathways involved in the regulation of beta-cell differentiation.

Materials and Methods: The human pre beta-cell line Blox5Pdx, which express Pdx-1 after Pdx-1 gene insertion, and the sub-population Blox5NI, which albeit the Pdx-1 gene is inserted does not differentiate, were studied for GLP-1 receptor (GLP1R) downstream signalling with 10 nM Ex4 during formation of pseudo-islets. Immunohistochemistry and western blotting were performed with antibodies against phosphorylated or non-phosphorylated p44/42MAPK, JNK/SAPK MAPK or p38MAPK, in combination with determination of insulin production by ELISA (detection limit 0.4 pM). Human pancreatic specimens were also studied with immunohistochemistry for phospho-p38MAPK in combination with insulin or the duct cell marker cytokeratin 19.

Results: Signalling through p44/42MAPK, JNK/SAPK MAPK and p38MAPK pathways were active after Ex4 induced differentiation of Blox5Pdx pseudo-islets. Following Ex4 stimulation of Blox5NI cells, p44/42 MAPK and JNK/SAPK MAPK signalling were also active, however, p38 MAPK was not activated. Inhibition of the GLP1R induced p38MAPK pathway by 10 nM SB203580 in Blox5Pdx cells during pseudo-islet formation, led to inhibition of insulin production and differentiation (<0.4 pM/24h with SB203580 vs. 287±25 pM/24h without). Moreover, activation of p38MAPK with 200 nM anisomycin in Blox5NI during GLP1R stimulation and pseudo-islet formation, partially restored insulin production (2.5±0.9 pM/24h vs. <0.4 pM/24h without anisomycin). We also found that in human pancreata the active form of p38MAPK is present only in insulin positive islet cells and in the ductal cells, which are believed to be the beta-cell precursors.

Conclusions: Activation of the p38MAPK pathway is required but not sufficient for insulin expression and pancreatic beta-cell differentiation in human pre beta-cells in vitro. Moreover, in human pancreas, activated p38MAPK is only associated with insulin producing cells and ductal beta-cell precursors.

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HNF3b is required for maintaining a-cell phenotype but not b-cell gene expression in differentiated islet cells

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Background and Aims: The HNF3 family of transcription factors, encoded by three genes HNF3a, HNF3b, and HNF3g, is necessary for the development and differentiation of the pancreas and liver. HNF3a and HNF3g regulate glucose homeostasis by activation respectively of pancreatic glucagon and hepatic gluconeogenic enzymes. HNF3b, which is expressed in islets, has been suggested as the upstream transactivator of HNF4a, HNF1a, Pdx1, and NeuroD/beta2, in the transcriptional hierarchy. The present study was designed to evaluate the role of HNF3b in regulation of pancreatic gene expression in differentiated islet cells.

Methods: The tetracycline-inducible system was employed to achieve tightly controlled expression of either HNF3b or its dominant-negative mutant, DN-HNF3b, in INS-1-derived INSrab clone showing both a- and b-cell phenotypes. Quantitative Northern blotting was performed using total RNA isolated from INSrab stable clones expressing HNF3b or DN-HNF3b. Cells were cultured in 2.5 mM glucose medium with or without 500 ng/ml doxycycline for 24 h, and continued for 8 h in 2.5, 6, 12, or 24 mM glucose medium. **Results:** After induction of DN-HNF3b, the glucagon mRNA level was reduced by 90%, whereas the expression of b-cell specific genes, insulin, IAPP, GLUT2 and Nkx6.1 remained constant. The expression of HNF4a, HNF1a, Pdx1, NeuroD/beta2, Nkx2.2, Pax4, and Pax6, was not altered by induction of DN-HNF3b, while the Isl-1 transcript level was decreased by 90%. In addition, HNF3b function was not required for the gene expression of the glycolytic enzymes, glucokinase, L-pyruvate kinase (L-PK), aldolase B, and GAPDH, and mitochondrial proteins, citrate synthase, ANT1, and ANT2. In contrast, overexpression of HNF3b in INSrab cells increased the glucagon mRNA level by two-fold. On the other hand induction of HNF3b suppressed the expression of the b-cell specific genes, insulin and IAPP by 50% and 60%, respectively. The mRNA levels of GLUT2 and glucokinase, which are implicated in glucose-sensing, were reduced by 90%, and 80%, respectively, after induction of HNF3b. As a result, the glucose-responsiveness of L-PK and aldolase B mRNA expression was blunted by overexpression of HNF3b. However, induction of HNF3b drastically raised the aldolase B mRNA level at 2.5 and 6 mM glucose. It also caused a 3 fold increase in the mRNA level of C/EBPb and 90% reduction in the expression of HNF4a and HNF1a. **Conclusions:** HNF3b is required for maintaining a-cell phenotype in differentiated islet cells. Overexpression of HNF3b promotes activation of glucagon and suppresses b-cell gene expression and glucose-sensing.

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The effects of nicotinamide and dexamethasone on beta-cell maturation.

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Background and Aims: Although a lot has been learned on the molecular mechanisms responsible for the differentiation of endocrine pancreatic cells, the important steps leading to full exocytotic maturity are not understood. The aim of this study was to find out which beta-cell or islet specific gene expression changes are associated with nicotinamide- and dexamethasone-induced differentiation of porcine fetal pancreatic cells.

Methods: Fetal pig pancreases (gestational age 90±5 days) were digested with collagenase and cultured for 14 days in RPMI1640 medium containing human serum and either 10 mM nicotinamide (NA) or 200 ng/ml dexamethasone (DXM). Insulin release from the islet-like cell clusters was studied with perfusion. For analysis of the gene expression profile we developed a cDNA dot-array method, allowing the simultaneous hybridization of labelled RNA samples (5 µg of total RNA) with 48 probes of interest in duplicate.

Results: Insulin expression was not maintained in culture while glucagon and somatostatin were upregulated in the absence of NA or DXM. Both NA and DXM potentially increased the insulin content, but only NA induced functional maturation in terms of glucose-sensitive insulin release. Insulin mRNA was increased 200-fold by NA and 70-fold by DXM. NA, unlike DXM, also upregulated SUR1, glucokinase, pdx-1 and Nkx 6.1 mRNAs. HES-1 (known as a suppressor of endocrine differentiation) was clearly detectable after DXM treatment but not in the other samples. A low level expression of the exocrine transcription factor p48 was maintained only in the presence of DXM. Reg expression was fully downregulated after NA but highly expressed after DXM treatment.

Conclusion: NA-induced functional beta-cell maturation, in contrast with DXM-induced upregulation of insulin mRNA without functional maturation, provides a model for the profiling of gene expression in order to gain understanding of the molecular mechanisms controlling beta-cell maturation.

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Critical Factors for the Endocrine Differentiation of Cultured Adult Human Ductal Cells

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Background and Aims: Shortage of donor organs is the major limitation in the development of beta-cell transplantation therapies for diabetes. It was recently reported that it is possible to induce endocrine differentiation from the ductal epithelial cells. Here we have reproduced the published method and aim to define the critical determinants required for endocrine differentiation in the adult human pancreatic ductal cell culture. **Materials and Methods:** Fractions of six human pancreatic digests were shipped from Uppsala and used for these studies. After initial attachment and monolayer expansion in the presence of serum, the cells were transferred to serum free medium (DMEM/F12 supplemented with 10ng/ml FGF-7 and 10mM nicotinamide). The cells were then overlaid with Matrigel. Cells were harvested for analysis after a total of 4-5 weeks in culture. **Results:** The original tissue contained 50±3% ductal (CK19-positive) cells and 9±1% insulin positive cells. During monolayer expansion, the proportion of ductal cells increased to 76±5% while the insulin-positive cells decreased to 2±0.3%. We were able to reproduce the previously published findings by demonstrating the development of cyst-like structures through the Matrigel layer, with numerous small dense cultivated human islet buds/CHIBs' growing out of the cyst walls. The CHIBs are likely to represent newly formed islets, since they contained 19±2% insulin-, and 13±1% glucagon-positive cells and colocalization of the two hormones in many cells. There was no cyst development at all if the cells were maintained in serum-containing medium. Also the Matrigel overlay procedure was found to be absolutely necessary. Furthermore, if the ductal cells were allowed to grow on another matrix found to support their growth (the 804G matrix), this also prevented the development of CHIBs. Omission of nicotinamide from the medium decreased both the number of CHIBs and their insulin content per DNA by about 40%. **Conclusions:** Our studies confirm that it is possible to induce a low degree of endocrine differentiation from adult human pancreatic ductal cells in long-term tissue culture. Serum-free conditions are essential for this process, indicating that serum contains growth factors inhibiting differentiation.

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Islet, Pancreas and Kidney Transplantation

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Kidney-islet transplantation in patients with type I diabetes: twelve years clinical follow-up

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Background and aims. Remarkable success has recently been reported in patients with type I diabetes who underwent islet transplantation alone (Edmonton experience). The rate of success after kidney-islet transplant is still a topic of discussion (Islet International Registry report). The aims of our study were to analyze the clinical outcome of kidney-islet transplants during a long-term follow-up and to characterize the main factors affecting the islet function.

Materials and Methods. 45 islets transplantations were performed in IDDM patients simultaneously or after a kidney graft. In all cases the islets were isolated with the modified automated method and they were infused in the liver under local anaesthesia. The study was divided into 2 Eras (1st Era: 1989-1996, 23 cases; 2nd Era: 1998-2001: 22 cases), according to the following modifications which were included in the protocol beginning from 1998: analysis of [Ca²⁺]_i changes and monocyte chemoattractant protein-1 secretion as control of islets viability; low doses/withdrawal of steroids and use of metformin to reduce the insulin resistance. The immunosuppression was the same during the both Eras, except for steroids: ALG or ATG for induction, cyclosporine plus azathioprine or micofenolate mofetil for chronic therapy. The complications were: empysemic (3 cases), spontaneously solved; emphysema (2 cases), solved with transcatheter drainage; serum sick syndrome (1 case); kidney rejection (2 cases) solved with steroids bolus.

Results. Only patients who received >6,000 islet equivalent number/kg were considered (1st Era: 13 cases; 2nd Era: all cases). In all cases the fasting C-peptide after the islet transplant was > 1 ng/ml. 1st Era: the Exogenous Insulin requirement (EIR) decreased <50% of the pre transplant dose in 7% of cases and in 54% of cases the insulin therapy was stopped, but only 1 patient maintained the insulin independence for >2 years; the HbA_{1c} was 7.4±1 after 1 year. 2nd Era: the EIR decreased <50% of the pretransplant dose in 32% of cases and 59% of patients stopped insulin therapy. Among them 38% resumed insulin therapy, 8 patients are still insulin free; in 3 cases the insulin independence is lasting >2 years. The HbA_{1c} was 6.2±0.7 after 1 year (the difference between the 2 Eras was statistically significant).

Conclusion. The islet transplantation, associated with kidney, completely replaces the endocrine function of pancreas in IDDM patients in >50% of cases. The improvement of islet preparation quality and the reduction of insulin resistance seem to be factors affecting the clinical outcome and the lasting of function.

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Stable in-situ chimerism is a key to graft acceptance in MHC-compatible rat pancreas transplantation

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Background and Aims: "Chimerism" is still controversial concept in the regulation of graft acceptance or tolerance in organ transplantation. We examined what is essential in the regulation of pancreas graft acceptance in a MHC-compatible combination.

Materials and Methods: Diabetes-prone BB (DP-BB) rats (RT1u, RT7.1) were transplanted with pancreaticoduodenal (PD) grafts from MHC-compatible WF rats (RT1u, RT7.2). Time-course analyses (2w, 4w, and 120days postgrafting or on graft failure) of recipient spleens (representative of recipient systemic environment), recipient paraaortic lymph nodes (representative of recipient local environment close to the graft), and graft pancreatic lymph nodes (representative of graft environment) were made using multi-color flow cytometry including intracellular cytokine expression and MLR to donor WF.

Results: Without immunosuppression, transplanted recipients were found to have resulted in three groups: Group (I) graft acceptance (>120 days) with systemic (splenic) macrochimerism (n=9), (II) graft acceptance (>120 days) without systemic macrochimerism (n=6), and (III) autoimmune recurrence (rec.) + rejection (rej.) (n=8, graft survival; 45.0±26.7 days). In group (I), each time, flow cytometric analysis showed macrochimerism in each organ (on 120days: RT7.2+T/T=85%) with remarkable proliferation of donor-derived NKR-P1+TCRαβ+ (NKT) cells predominantly CD8+ (on 120days: in spleen NKT/T=15.7±15.7% vs 3.3% of naive-DP-BB; NKT/T=2.0±0.4% in paraaortic lymph nodes (on 120days: RT7.2+T/T=96.4±9.9% though no chimerism (<0.1%) was found in splenic T cells on 120days). In group (II), on rec.+rej., no chimerism (<0.1%) was found in all of the organs. In the pancreatic lymph nodes of the rec.+rej. recipients, recipient-derived NKT cells, predominantly CD8+, were remarkably proliferated (NKT/T=9.4±9.4% vs 2.8% of naive-DP-BB) and more than 40% of those NKT cells were intracellular IFN-γ+ and T cells of those pancreatic lymph nodes showed significantly high MLR response to WF, compared with those of the other organs and those of the other groups. Further quantitative PCR analyses of the lymphocytes in each organ in all groups in terms of chimerism and cytokine expression which support those results are being examined.

Conclusions: Our striking new concept "stable in-situ chimerism" with recipient chimeric paraaortic lymph node cells as a sentinel is a key to PD graft acceptance in MHC-compatible pancreas transplantation.

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REDUCTION OF BLOOD GLUCOSE VARIABILITY BY ISLET TRANSPLANTATION: INTEREST OF CONTINUOUS GLUCOSE MONITORING

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Background and Aims: To compare the glycemic profile of type I diabetic patients treated by an implantable insulin pump or a pancreas or an islet transplantation by the mean of the Continuous Glucose Monitoring System (CGMS, Minimed Inc, Sylmar, CA). **Materials and Methods:** The CGMS enabled to record subcutaneous concentrations of glucose (range 2.2-22 mmol/l) during 72 hours (288 measurements per day). These measurements needed to be validated by 4 capillary blood glucose per day (calibration). During 3 days, CGMS was connected to 19 type I diabetic patients: six patients (age: 49.8±7.4 years, diabetes duration: 31±11.3 years) were treated by intraperitoneal insulin infusion with implantable pump (group IPII), eight patients (age: 42.2±11.6 years, diabetes duration: 27.2±8.7 years) by simultaneous kidney pancreas transplantation (group SPK) and 5 patients (age: 43.3±13.6 years, diabetes duration: 26.6±12.5 years) by pancreatic islet transplantation after kidney graft (group IAK). All SPK patients and two IAK patient were insulin-independent, while three IAK patients had partial graft function and reduced exogenous insulin needs. Immunosuppression therapy of all transplanted patients associated cyclosporine, mycophenolate mofetil and steroid (0 to 5mg/l). Glucose control was evaluated by the measure of mean glucose concentrations and mean amplitude of glycemic excursions (MAGE index) during 3 days and by HbA_{1c}. Statistical analysis was performed by an ANOVA test followed by a Student-Newmann-Keuls test. **Results:** During 3 days, both mean glucose concentrations and mean amplitude of glycemic excursions in pancreas and islet transplanted patients were significantly lower than that observed in patients treated by IPII: 5.38±1.12 and 6.02±0.86 vs 8.06±1.03 mM (p<0.001) and 1.44±0.42 and 1.42±0.57 vs 3.46±0.71 mM (p<0.001), respectively. Furthermore, the mean glucose concentrations and the fluctuations in blood glucose concentrations were comparable in patients treated by pancreas or islet transplantation. HbA_{1c} in patients treated by pancreas or islet transplantation were 5.35±0.37% and 6.03±1.02%, respectively, while this value reached 7.21±0.55% in patients treated by IPII (p<0.001). **Conclusions:** continuous subcutaneous glucose monitoring suggests that islet transplantation can be as efficient as pancreas transplantation and superior to implantable insulin pump in restoring a good metabolic control and reducing blood glucose variability.

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SPECIFIC PATHOGEN FREE PIG ISLETS DO NOT TRANSMIT FULL LENGTH OR SUBGENOMIC RETROVIRAL SEQUENCES TO HUMAN CELLS, DURING IN VITRO COINCUBATIONS.

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Background and Aims: Pig islets may be grafted into Type 1 diabetic patients. The use of islets from specific pathogen-free (SPF) pigs could prevent transmission of conventional zoonosis' from pigs to humans, but not of pig endogenous retroviruses (PERV). The risk of PERV transmission may partly depend on the tissue to be grafted. Our previous work indicated that pancreas expressed the lowest levels of PERV mRNAs among pig tissues intended for grafting. The aim of the present work was to determine whether the presence of PERV group gamma-1 mRNAs in pig islets could produce infection of human cells during in vitro co-incubations.

Materials and Methods: Human cell lines (293, n=3; Jurkat, n=8; K562, n=1) or peripheral blood mononuclear cells (PBMC, n=2) were co-incubated with pig islet cells from SPF pigs, under conditions designed to increase the probability of contact between pig and human cells (high islet/human cell ratio, extended period of coculture and repeated contacts). Irradiated PK15 and G2 retrovirus-producing pig cell lines were used in place of islet cells as positive infection controls. Infection of human target cells was monitored both on cellular extracts and cell culture supernatants. PCR and long PCR were performed to detect PERV DNA, and RT-PCR and long RT-PCR to detect PERV mRNA. Additionally, PERT assays were used for detection of reverse transcriptase activity on the corresponding supernatants.

Results: Despite the presence of all PERV sequences, including full-length PERV inserts, in pig islet cells (n=6), no signals for gag, pol, and env of PERV, could be detected at DNA and RNA levels in any human cell line or PBMC co-incubated with pig islet cells. Supernatants also remained negative. These results were verified in all experimental coculture conditions tested. In contrast, full-length PERV inserts and PERV mRNA transcripts for gag, pol, and env were detected in the same human cells previously co-incubated with pig PK15 or G2 cell lines. PK15 cells transmitted env-A and -B, whereas G2 cells transmitted env-C, in addition to env-A and -B.

Conclusions: These results suggest that SPF pig islet cells, despite full-length PERV inserts and transcription of PERV sequences, do not transmit PERV sequences to human cells during in vitro co-incubation under the present experimental conditions.

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PRESERVED LONG-TERM BETA CELL FUNCTION AND GLUCOSE TURNOVER IN KIDNEY-PANCREAS TRANSPLANT PATIENTS
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Background and Aims: Patients with diabetes undergoing kidney-pancreas transplantation are treated with immunosuppressive drugs, which potentially deteriorate beta cell function and insulin sensitivity. The present study was undertaken to test the long-term beta cell capacity as well as glucose tolerance and turnover in recipients with functioning kidney-pancreas grafts. **Materials and Methods:** Seven diabetic patients with functioning kidney-pancreas allografts (group 1) were compared to 8 non-diabetic patients with a normal intravenous glucose tolerance test (IVGTT) and a functioning single kidney allograft (group 2) 2-9 years after transplantation. The two groups received similar doses of immunosuppressive drugs (prednisolone 7.5 vs. 8.0 mg/day and CyA 225 vs. 231 mg/day). Both groups were examined by an IVGTT and a euglycemic hyperglycemic clamp (EHC) designed to half-maximally suppress endogenous glucose production. Total glucose turnover was measured by $3\text{-}^3\text{H}$ -glucose added to the glucose infusate. **Results:** The glucose excursions after the IVGTT were identical in group 1 and 2 (AUC 1016 ± 30 vs. 981 ± 56 mM (n.s., SEM). First and second phase insulin release were significantly higher in group 1 compared to group 2 (1st phase 12.8 ± 0.8 vs. 9.2 ± 0.7 nM, 2nd phase 44.5 ± 6.7 vs. 22.4 ± 8.1 nM, $p<0.01$ for both comparisons). However, AUC C-peptide was identical (352 ± 68 vs. 426 ± 45 nM). During EHC the average plasma insulin was higher in group 1 than in group 2 (390 ± 68 vs. 195 ± 32 pmol/l, $p<0.01$). Despite this the two groups were identical in terms of peripheral glucose uptake (17.4 ± 1.4 vs. 16.2 ± 1.3 $\mu\text{mol/kg/min}$), and percent suppression of endogenous glucose production (63 ± 4 vs. $58\pm3\%$) and free fatty acids (73 ± 9 vs. $69\pm8\%$). Due to hyperinsulinemia the insulin sensitivity index for glucose uptake in group 1 was only half of that in group 2 (0.04 ± 0.003 vs. 0.09 ± 0.01 , $p<0.01$). **Conclusions:** Diabetic patients with functioning pancreas allografts have, despite significant hyperinsulinemia, a glucose turnover identical to normoglycemic non-diabetic renal transplant patients. Insulin sensitivity is accordingly down-regulated. Since C-peptide release is not elevated, the hyperinsulinemia in patients with pancreas allografts is probably caused by the absence of first pass liver uptake of insulin.

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Course of diabetic retinopathy and polyneuropathy after successful simultaneous pancreas and kidney transplantation: Results of long-term follow-up
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Background and Aims: Simultaneous pancreas and kidney transplantation (SPK) is performed in patients with advanced forms of microvascular disease, whose modulation by induction of normoglycemia has become subject of intensive research. We report preliminary results of follow-up of diabetic retinopathy (DR) and polyneuropathy (PN) after SPK.

Materials and Methods: We examined 118 subjects with Type I diabetes divided into two subgroups: (1) patients after successful SPK - SPK group (n=67, mean follow-up period of 54 months), (2) patients with a functioning kidney graft only - K group (n=51, follow-up period 73 months). The patients had an ophthalmologic examination including visual acuity, funduscopy, and assessment of the need of laser therapy. Neurological examination included electromyography (EMG) and clinical assessment of grade of PN. The examinations were performed before transplantation (Tx) and at the end of follow-up (minimum one year). Results after 3 years were available in 72 patients; additionally, DR could be assessed in 19 patients 5 years post-Tx.

Results: Mean (\pm SD) visual acuity in the SPK group improved slightly, albeit non-significantly (the values at baseline and at the end of follow-up were 0.43 ± 0.37 and 0.44 ± 0.36 , respectively). A non-significant deterioration of results occurred in the K group (0.46 ± 0.36 and 0.33 ± 0.32). However, an inter-group comparison revealed a significantly different course of visual acuity between the groups post-Tx (SPK vs K $p<0.01$). This trend was also evident in the group of patients with a documented 5-year follow-up ($p<0.05$). Funduscopy at the end of follow-up showed more findings of stabilisation or improvement of DR in the SPK group compared with the K group ($p<0.001$). Similar results were obtained when assessing the findings at 3 years post-Tx. The post-Tx course was associated with a significantly lower need of laser therapy in the SPK than in the K group (30.7 vs 57.5% , $p<0.001$). At the end of follow-up, EMG of the n.medianus showed an improvement of nerve conduction velocity in the SPK group in comparison with the K group where a decrease was demonstrated ($p<0.05$). The difference was evident after 3 years of follow-up ($p<0.05$). An improvement of the results of the clinical assessment of PN occurred in the SPK group whereas the findings were unaltered in the K group. The inter-group difference was significant ($p<0.01$).

Conclusions: The results of the study suggest that pancreas Tx has beneficial effect on the course of diabetic retinopathy and polyneuropathy. Supported by grant ND 5295-3 IGA MZCR.

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NORMALIZATION OF PLATELETS CHRONIC ACTIVATION AFTER KIDNEY-PANCREAS TRANSPLANTATION IN UREMIC TYPE 1 DIABETIC PATIENTS. A POSSIBLE ROLE FOR PAR1-PAR3 RECEPTOR
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Background and Aims: Platelet hyperfunction is a typical feature of chronic complications of diabetes. Diabetic uremic patients are at high risk of cardiovascular mortality, with a tendency to a prothrombotic state. Intracellular ionized calcium is involved in the mechanism of signal transduction that lead to platelets activation. Previous studies showed that platelets' calcium homeostasis is impaired in uremic type 1 diabetic patients in the directions of a chronic activation. Our aim was to show the effect of kidney-pancreas transplantation (by restoring euglycemia and normalisation of uremic state) on platelets' calcium homeostasis and the underlying mechanisms.

Materials and Methods: 20 uremic type 1 diabetic patients, enrolled from our waiting list, matched for age, sex, diabetes duration and dialysis duration underwent kidney-pancreas transplantation (KP, n=11) or remained on dialysis [diabetic uremic (WL), n=9]. Blood platelets were processed and loaded with Fura-2 to study intracellular ionized calcium ($[\text{Ca}^{2+}]_i$) both in resting condition and after a stimulus with 0.5, 0.1 and 0.05 U human thrombin respectively. The ($[\text{Ca}^{2+}]_i$) levels were analyzed in resting condition, as peak after stimulus and plateau. Moreover, platelets underwent FACS analysis to study receptor expression of P-selectin (CD63), GpIIb/IIIa (CD62), PAR1 and PAR3 receptor.

Results: Resting ($[\text{Ca}^{2+}]_i$), the most sensible index of basal platelet activation, was higher in WL than in KP patients (WL = 134.0 ± 8.1 vs KP = 96.6 ± 9.1 , $p<0.01$). The peak after the stimulus was higher in KP than in WL (at 0.5 U: WL = 774.0 ± 101.3 vs KP = 1332.1 ± 267.9 , $p=0.09$; at 0.1 U: WL = 478.2 ± 56.8 vs. KP = 803.3 ± 89.9 , $p=0.02$; at 0.05 U: WL = 429.6 ± 42.5 vs KP = 600.4 ± 75.3 , $p=0.08$). No differences were evident for the plateau. Finally, it was observed that a different expression of PAR1 and PAR3 expression was evident in the 2 groups of patients.

Conclusions: A possible role for this pathway should be claimed in the derangement of platelets calcium homeostasis in uremic type 1 diabetic patients and in the normalization, which occur in KP group. Restoring of euglycemia and normalization of uremia is able to normalize almost all platelet alterations evident in uremic type 1 diabetic patients. Particularly, chronic platelets activation seems to be normalized after kidney-pancreas transplantation.

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CARDIOVASCULAR (CV) RISK FACTORS (RF) IN RECIPIENTS OF SUCCESSFUL KIDNEY-PANCREAS OR KIDNEY TRANSPLANTATION.
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Background and Aims: Cardiovascular disease (CD) is the main cause of morbidity and mortality in patients with organ transplantation, mainly due to the high prevalence of several RF in these patients.

Materials and Methods: We evaluated and compared the prevalence of a number of RF for CD in 35 previously diabetic recipients of functioning kidney-pancreas graft (KPG, age 42 ± 9 yrs, BMI 23.9 ± 2.3 kg/m², M/F 20/15) and in 99 non-diabetic recipients of functioning kidney graft (KG, age 44 ± 10 yrs, BMI 24.8 ± 3.8 kg/m², M/F 23/12). The two groups were on stable immunosuppressive therapy, based on azathioprine, prednisone and cyclosporine in the KG group, and MMF, prednisone and cyclosporine in the KPG group.

Results: In the KPG group, a significant improvement of: fasting plasma glucose (87 ± 11 vs 230 ± 33 mg/dl, $p<0.001$), HbA1c (5.5 ± 0.6 vs $8.7\pm1.1\%$, $p<0.001$), total cholesterol (192 ± 37 vs 223 ± 48 mg/dl, $p<0.001$), LDL-cholesterol (112 ± 26 vs 123 ± 35 $p<0.05$), triglycerides (113 ± 29 vs 226 ± 81 , $p<0.001$), systolic (Syst-BP) (125 ± 14 vs 154 ± 20 mmHg, $p<0.05$) and diastolic (Dyast-BP) (76 ± 8 vs 90 ± 8 mmHg, $p<0.02$) blood pressure was observed after transplantation. In KG, total cholesterol (247 ± 52 mg/dl, $p<0.001$), LDL-cholesterol (152 ± 40 mg/dl, $p<0.001$), triglycerides (181 ± 88 mg/dl, $p<0.001$) and fasting plasma glucose (96.9 ± 14 mg/dl, $p=0.035$) and blood pressure values (Syst-BP 138 ± 13 $p<0.05$) (Dyast-BP 85 ± 9 , $p<0.05$) were significantly higher than in KPG patients.

Conclusions: These results show that recipients of kidney-pancreas graft show reduced post-Tx CV RF; despite the previous presence of diabetes, this group has a risk factor profile better than non-diabetic recipients of kidney graft.

OP 47 Autoimmunity

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ANTI-CD38 AUTOANTIBODIES: NEW MARKERS OF BETA CELL AUTOIMMUNITY IN TYPE 1 AND TYPE 2 DIABETES

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Background and Aims: insulin secretion is one of the functions mediated by CD38, a lymphocytic ecto-enzyme capable of mobilizing Ca^{2+} through cyclic ADP-ribose production. The molecule is the target of an autoimmune response, since serum anti-CD38 autoantibodies (aAbs) have been detected in diabetic patients. We asked whether the target molecule is also expressed in the human pancreas and undertook a comprehensive analysis of anti-CD38 aAbs. **Materials and Methods:** an immunohistochemical analysis of CD38 in the human pancreas was performed; anti-CD38 aAb prevalence in type 1 and type 2 diabetic subjects as well as in patients classified as Latent Autoimmune Diabetes in the Adult (LADA) was analyzed by a novel enzymatic immunoassay developed to this aim. **Results:** CD38 was found to be selectively expressed in a functionally active form in human islets of Langerhans, with a molecular weight of approx. 48 kDa vs. the conventional 45 kDa. Anti-CD38 aAbs were detected in 19.2% of type 1 and 16.7% of type 2 diabetic subjects, as compared with a 1.5% prevalence among healthy controls matched for age and BMI. LADA patients displayed a prevalence of anti-CD38 aAbs of 10.9%. The majority of anti-CD38 aAbs (57.1%) revealed agonistic properties, inducing Ca^{2+} mobilization in lymphocytes and insulin secretion in purified islet cells. In agreement with these functional features, the presence of anti-CD38 aAbs was associated with significantly higher levels of fasting plasma C-peptide and insulin in type 2 patients, along with higher BMI values and a lower frequency of insulin therapy. There was no association between anti-CD38 aAbs and other conventional markers of beta cell autoimmunity (anti-GAD, ICA, anti-IA-2), neither among type 1 nor among type 2 patients. **Conclusions:** these results underline the significance of anti-CD38 aAbs as new markers of beta cell autoimmunity, both in type 1 and type 2 diabetes, marking a subset of patients similar to LADA subjects for the autoimmune features but characterized by higher residual beta cell function. Moreover, the prevalent agonistic activity of anti-CD38 aAbs suggests their potential contribution to the pathogenesis of the disease, possibly damaging beta cells (exhaustion from overstimulation) and other CD38⁺ tissues (effects mediated by Ca^{2+} mobilization).

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Soluble adhesion molecules in preclinical type 1 diabetes - a longitudinal study
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Background and Aims: The role of cell adhesion molecules in the pathogenesis of Type 1 diabetes has been characterized in experimental models of autoimmune diabetes revealing strong expression of intercellular adhesion molecule-1 (ICAM-1) by islet-infiltrating lymphocytes. Increased levels of soluble adhesion molecules in the circulation, shed from the cell surface, could be an epiphenomenon of immune activation and thus might provide a useful monitor of disease activity. This prospective case-control study aimed at evaluating the time-course of serum concentrations of soluble ICAM-1 and L-selectin in siblings of affected children with signs of preclinical Type 1 diabetes to assess whether these markers could discriminate between those siblings who progressed to clinical diabetes and those who remained non-diabetic.

Materials and Methods: Serum levels of ICAM-1 and L-selectin were measured by specific enzyme-linked immunosorbent assays in 30 autoantibody-positive initially healthy siblings of diabetic children who progressed to clinical disease during the observation period of 10 years and in 30 sex and age-matched autoantibody-positive siblings who have remained unaffected.

Results: Integrated concentrations (area under the curve) of sICAM-1 over a period of 48-6 months before the diagnosis was significantly higher in the progressors ($p=0.035$), the difference being most evident 1.5 years before diagnosis ($p=0.005$). The integrated concentrations of soluble L-selectin were similar in progressors and non-progressors over the total preclinical period.

Conclusions: The present study suggests that the process of destructive insulinitis may be initiated on an average approximately 4 years before the manifestation of clinical diabetes, being most active 1.5 years before the diagnosis. Peripheral concentrations of soluble ICAM-1 or L-selectin are not helpful in the identification of those prediabetic subjects who will progress to clinical disease over the next 10 years, since there is substantial overlapping in these concentrations between progressors and non-progressors.

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REDUCED EXPRESSION OF TH1 ASSOCIATED CHEMOKINE RECEPTORS ON PERIPHERAL BLOOD LYMPHOCYTES OF PATIENTS WITH NEWLY DIAGNOSED TYPE 1 DIABETES

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Background and Aims: In the last years a new class of receptors on blood cells, chemokine receptors, has been identified. They govern the migration of cells to inflamed tissue. In the T cell mediated autoimmune disease multiple sclerosis chemokine receptor expression on T cells has been suggested as a surrogate marker for immune activity. No such studies are available for type 1 diabetes.

Materials and Methods: We have investigated 25 patients with newly diagnosed type 1 diabetes (NDD) at diagnosis, 10 patients with longstanding type 1 diabetes (1 to 5 years diabetes), 10 patients with newly diagnosed type 2 diabetes and 35 matched healthy controls for expression of chemokine receptors CXCR4 (expressed on naive T cells), CCR5 and CXCR3 (expressed on TH1 T cells) and CCR1 and CCR2 (expressed on TH2 T cells). Chemokine receptor expression was measured using FACS analysis after triple colour staining. Chemokine serum levels (MIP-1a, MCP-1, RANTES) were measured by commercial ELISA's.

Results: TH1 associated T cells were significantly reduced in patients with newly diagnosed type 1 diabetes when compared to matched controls ($p<0.01$ for CCR5, $p<0.02$ for CXCR3). These differences were not observed in patients with longstanding type 1 diabetes or type 2 diabetes and reversed 3 to 6 months after diagnosis. We found no relevant expression of TH2 associated chemokine receptors in any group (generally $<2\%$ of T cells). Reduced TH1 associated receptor expression on peripheral T cells correlated to reduced PHA-stimulated TH1 cytokines (IFN γ , TNF α). In a subgroup of NDD patients, MIP-1a levels were elevated while we found no difference in MCP-1 or RANTES serum levels compared to controls.

Conclusions: We hypothesize that at time of diagnosis of type 1 diabetes TH1 cells migrate to the pancreas. Chemokine receptor expression on peripheral T cells may be a useful surrogate marker for natural history of immune activity in type 1 diabetes.

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DIABETES ANTIBODY STANDARDISATION PROGRAMME: FIRST ASSAY PROFICIENCY EVALUATION

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Background and Aims: The Diabetes Antibody Standardisation Programme (DASP), an extension of Immunology of Diabetes Society autoantibody workshop activities, was established to evaluate and improve general implementation of assay methods, and to undertake extended evaluation of the new WHO international reference reagent for GADA and IA-2A.

Materials and Methods: Forty-three laboratories in 15 countries registered for the first proficiency evaluation. Participants received coded sera from 50 patients with newly diagnosed type 1 diabetes (median age 17, range 9-29 years) and 50 blood donor controls, together with the WHO reference reagent and diluent serum. Results were analysed using receiver operator characteristic (ROC) curves. Sensitivity was adjusted to 90% specificity in workshop controls.

Results: The median adjusted sensitivity for GADA (41 laboratories) was 85% (range 68-96%), for IA-2A (40 laboratories) was 58% (24-86%) and for IAA (20 laboratories) was 34% (6-66%). ROC curve analysis showed that all GADA assays, 39/40 IA-2A assays and 15/20 IAA assays found significant differences between patients and controls. There was good concordance between laboratories in ranking of samples according to GADA and IA-2A levels, or if results were expressed in relation to the common standard. Assays achieving the highest sensitivity for IAA were also concordant in ranking samples but overall concordance for IAA was poor.

Conclusions: GADA and IA-2A assays generally achieve high sensitivity and specificity, and inter-laboratory concordance is good when results are expressed in common units based on the WHO reference reagent. The sensitivity of IAA assays in most laboratories is low, but there are IAA microassays that achieve high sensitivity and good concordance. Differences in assay protocols between laboratories must be addressed so that all centres can perform to the same high standard.

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INSULIN AUTOANTIBODY ISOTYPES IN CHILDREN WITH INCREASED GENETIC RISK FOR TYPE 1 DIABETES

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Background and Aims: The isotype profile of antigen specific autoantibodies may reflect either a Th1 or Th2 immune response. This study aimed at assessing the maturation of the humoral immune response to insulin in preclinical type 1 diabetes by timing the emergence of various isotypes of insulin autoantibodies (IAA) after the initial appearance of such antibodies in genetically susceptible children identified from the general population.

Materials and Methods: The study population is derived from the Finnish DiPP Study that observes children with HLA-conferred genetic risk from birth for signs of beta-cell autoimmunity. Blood samples were obtained with an interval of 3-6 months over the first 2 years and subsequently with an interval of 6-12 months. The present series includes the first 45 children who have seroconverted to IAA positivity. Fifteen have so far presented with type 1 diabetes (progressors), while 30 have remained non-diabetic (non-progressors). An isotype specific radiobinding assay was used to determine isotype-specific IAA (IgG1-4 and IgA). The integrated antibody levels over the observation period were assessed by analyzing the area-under-the-curve (AUC).

Results: The progressors had an IgG1 response to IAA in their first positive sample more often than the non-progressors (14/15 vs. 20/30; $p=0.05$). Nine progressors had initially a dominating IgG1 response, five an IgG3 response, and one another isotype (IgG4), whereas the corresponding distribution among the non-progressors was 25/0/5 ($p=0.003$). The progressors had higher integrated levels of IgG1 ($p=0.04$) and IgG3 ($p=0.002$) subclass IAA. Eight progressors had a dominating integrated IgG1 response, six a dominating IgG3 response and one a dominating IgG4 response over the observation period, while 22 non-progressors had a dominating IgG1 response, five an IgG2 response, one an IgG3 response and one a dominating IgG4 response ($p=0.008$). Half of both the progressors and non-progressors had a detectable IgG4 response during the follow-up period.

Conclusions: These data show that genetically susceptible young children who progress to clinical Type 1 diabetes are characterized by a strong IgG1 and IgG3 response to insulin. A weak or lacking IgG3 response is associated with relative protection against Type 1 diabetes, since one third of the non-progressors had no detectable IgG3 response during follow-up and an IgG3 response.

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Incidence of childhood type-1-diabetes in Denmark

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Background and Aims: The incidence rate of childhood diabetes has increased in Europe and many other parts of the world over the past 50 years Denmark belongs to a high incidence area. Former studies have suggested increasing incidence in males until 1970. Since 1996 Denmark have had a national register. This is a report of these results comparing with earlier reports. The aims is therefore 1) to determine the incidence of type-1-diabetes in Danish children 1996-1999. 2) To evaluate trends in incidence and age at onset from 1970-1999.

Materials and Methods: The incidence rates for type-1-diabetes in Denmark (1996-99) were obtained from the National childhood diabetes register. The age-specific incidence rates are compared with data collected retrospectively in 1970-76 and data from 1980-84, both studies representing population based studies using existing national routine registration of hospital admissions to paediatric departments within the survey area. Population data were obtained from Statistics Denmark. The statistical method used is a Poisson regression model:

$\text{Log}(\text{Incidence-rate}) = A + B(\text{age,gender}) + D(\text{county}) + E(\text{age}) \cdot \text{birthcohort}$

Results: The annual incidence in 1996-1999 in Denmark is 12.5 in the age group 0-4 years-of-age; 17.2 in the age group 5-9 years-of-age and 24.5 in the age group 10-14 years-of-age. There is a significant increase in incidence in the age group 0-4 years-of-age 1.8% (0.67-3.0%) per year, and there is a significant increase in the age group 5-9 years-of-age 1.1% (0.13%-2.0%) per year. In the oldest age group the annual incidence increase is 0.23% (-0.6-1.1%). The three time trends are different ($P=0.043$). The annual increase in incidence is 0.8% (0.21-1.49%) for the age group 0-14 years-of-age.

Conclusions: There is in Denmark - as in other European countries - a left shift in age of onset and an annual increase in the total age group 0-14 years-of-age.

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Incidence of type 1 diabetes in young adults across Europe - an EU funded multicentre study (IDA)

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Background and Aims: The epidemiology of the childhood onset form of Type 1 diabetes (i.e., ages < 15 years) has been studied extensively. In contrast, the epidemiology of the adult onset form of the disease is poorly characterized. Reports from a small number of European countries have suggested a higher incidence in males than in females in the adults compared to the children where there is either no gender difference or a slight excess female incidence rate. The aim of this study was to investigate the geographical distribution of sex and age specific incidence of Type 1 diabetes with onset in young adulthood in Europe. **Materials and Methods:** Nine centres located all over Europe took part in the study: Sardinia (Italy), Romania, Catalonia (Spain), Slovakia, Antwerp region (Belgium), Lithuania, Sweden, Yorkshire and Leicestershire (UK). Incident cases of type 1 diabetes in the age group 15-29 years were collected by a standardized questionnaire and reported to a central co-ordinating office along with demographic data. Incidence rates were estimated as cases per 100 000 person year and standardized for age and sex. **Results:** The standardized incidence for 1996-97 varied from 4.9/100 000 person years in Slovakia to 13.4/100 000 person years in Leicestershire. Sweden and Sardinia, known to have very high childhood incidences, had incidences less than half of those for children. Contrary to this is for example Romania, with a childhood incidence of 5.3/100 000 and an adult incidence of 6.6/ 100 000. The male-female ratio was ≥ 1 in all centres and in the age group 25-29 years, it was ≥ 1.5 . **Conclusions:** The range of the incidence of type 1 diabetes in adults is smaller than in children. In the two centres with the highest childhood incidence it was markedly lower in young adults. This could indicate that the rise in childhood incidence described in studies such as the EURODIAB TIGER, could be limited to certain age groups, although a longer follow-up is needed. Furthermore, there is a male excess in incidence, especially in the age group 25-29 years, in all eight European countries taking part in this study. Whether this can be explained by biological differences between the sexes remains to be solved.

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Variation at the insulin VNTR locus but not the CTLA-4 locus is associated with age of onset and need for insulin therapy in latent autoimmune diabetes in adults (LADA)

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Background and Aims: Subjects with Latent autoimmune diabetes in adults (LADA) have islet cell antibodies (ICA) and glutamic acid decarboxylase antibodies (GADA) typical of Type 1 diabetes. Disease presentation is more typical of Type 2 diabetes. Decreased disease severity in LADA patients may be related to reduced genetic susceptibility. To determine if there is an association of the Type 1 diabetes susceptibility genes, the insulin VNTR (11p15.5) and CTLA-4 (2q33) with LADA, the allele frequencies in LADA and Type 2 diabetic patients were compared. Genetic variation at the insulin VNTR and CTLA-4 loci was studied in relation to phenotypic characteristics. **Materials and Methods:** Patients examined included 373 LADA, 530 Type 2 diabetic patients from the UKPDS and 330 non-diabetic subjects from the Diabetes in Families (DIF) study. Patients were genotyped by PCR-RFLP for the -23HphI A/B polymorphism in the insulin gene (which is in linkage disequilibrium with the VNTR class I/III alleles) and for the A49G transition in exon 1 of CTLA-4. **Results:** The VNTR class I alleles were significantly associated with LADA compared to Type 2 diabetic patients ($p<0.0001$). Division of patients by age at diagnosis showed a significant association of the VNTR class I alleles in those presenting younger: $p<0.0001$ (25-<35 years), and $p<0.0001$ (35-<45 years). The frequency of the VNTR class III alleles was increased in the older age groups (45-<55 and 55-65 years). In those diagnosed between 55 and 65 years, the class III allele frequency was similar to that in the non-diabetic subjects. There was an association of VNTR class I alleles with age at diagnosis ($p=0.026$), BMI ($p=0.009$) and need for insulin therapy within 6 years of diagnosis ($p=0.03$). There was no association of the CTLA-4 polymorphism with LADA. **Conclusions:** Genetic susceptibility from the insulin VNTR locus is associated with a younger age at diagnosis and a more severe phenotype in LADA patients suggesting a similarity between younger LADA and Type 1 diabetic patients.

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Nephropathy: Genetics and Pathology

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Increase in mesangial and endothelial cells but no change in podocyte number in type 1 diabetic patients with nephropathy.

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Background and Aims: Recent studies in both type 1 and type 2 diabetes have suggested that low podocyte number may be linked to the development of albuminuria. In these studies, glomerular cell number was estimated using the indirect method of Weibel. We set out to explore this issue using the unbiased disector principle, which is the current gold standard methodology.

Materials and Methods: Renal biopsies were analysed from 24 type 1 diabetic patients, mean (range) age 42 (20-59) years; median (range) albuminuria 198 (30-1599) µg/min; GFR 88 (57-135) ml/min; and blood pressure 124 (97-147) / 74 (61-88) mmHg, and compared to 10 non-diabetic controls, age 38 (22-60) years. Light microscopy was used to estimate glomerular cell number by the unbiased disector/fractionator method and mean glomerular volume by the Cavalieri principle. Capillary surface area and length were estimated from electron micrographs.

Results: Both the number of mesangial cells and endothelial cells were significantly increased in the diabetic patients compared to controls (mean ± sd: 1468 ± 700 v 723 ± 250, $p < 0.001$; 1344 ± 454 v 934 ± 240, $p = 0.002$, respectively). There was no difference in the number of podocytes (482 ± 149 v 539 ± 149, $p = 0.318$). Mean glomerular volume was increased in the diabetic patients compared to controls (4.2 ± 1.3 v 3.2 ± 1.1 x 106 µm³, $p = 0.036$). Surface covered by podocytes (filtration surface + mesangio-urinary surface) was not different, however (0.47 ± 0.15 v 0.55 ± 0.11 mm², $p = 0.157$). Also, there was no difference in capillary length between the groups (29 ± 9 v 26 ± 6 mm, $p = 0.232$). The number of mesangial cells correlated with albuminuria ($r = 0.50$, $p = 0.012$). There was no correlation between the number of podocytes and albuminuria.

Conclusions: In early type 1 diabetic nephropathy there is no apparent reduction in the number of podocytes. The observed increase in glomerular volume appears to be in response to an increase in solid elements - cells and matrix - with no accompanying increase in filtration surface or capillary length.

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CONTRIBUTION OF eNOS GENE POLYMORPHISM (Glu298Asp) TO THE DEVELOPMENT OF DIABETIC NEPHROPATHY

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Background and aims: The contribution of endothelial nitric oxide synthase (eNOS) gene polymorphism (Glu298Asp) in exon 7 to the development of diabetic nephropathy was studied longitudinally in 116 type 1 diabetic patients (observation period: 22 ± 9 years). **Methods:** eNOS gene polymorphism was identified by PCR-RFLP method. The presence of albuminuria ≥ 30 mg/gCre at consecutive determinations was defined as the development of microalbuminuria. The patient's mean HbA1c (mHbA1c) values since the onset of diabetes was regarded as the index of long-term metabolic control. **Results:** The patients with missense variant (n=27) progressed to the occurrence of microalbuminuria earlier than those without missense variant (n=89) (incidence rate; 7.46 vs. 4.32 /100 patient-years, $p=0.02$). There was no difference in mean HbA1c values and frequency of hypertension between these two groups. However, in Cox's proportional hazard model, an independent risk factor for the development of microalbuminuria is not missense variant of eNOS gene but mHbA1c value (hazard ratio: 1.51/1% mHbA1c change, $p=0.001$). Then, the patients were subdivided to the better of glycemic control-group (mHbA1c levels < 8.5%, n=60) and the worse glycemic control-group (mHbA1c levels ≥ 8.5%, n=58). In the better glycemic control-group, the difference of cumulative incidence rate of microalbuminuria was conspicuous between the patients with missense variant of eNOS gene and normal variant of this gene (incidence rate; 6.90 vs. 2.44 /100 patient-years, $p=0.006$). In this group, Cox's proportional hazard model showed that missense variant of eNOS gene was an independent risk factor (hazard ratio: 3.13, $p=0.008$). On the contrary, in worse glycemic control-group, there was no difference in the cumulative incidence rate of microalbuminuria between the patients with missense variant and those without. **Conclusion:** eNOS gene polymorphism (Glu298Asp) contributes to the development of microalbuminuria, especially in the diabetic patients with mild to moderate hyperglycemia.

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THE ROLE OF HAEMOCHROMATOSIS C282Y AND H63D MUTATIONS IN DEVELOPMENT OF DIABETIC NEPHROPATHY

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Background and Aims: In patients with clinical haemochromatosis the frequency of diabetes ranges from 20 to 50% and the heterozygosity for the C282Y mutation in the HFE gene might be associated with increased risk for diabetes mellitus. There were also some reports suggesting that iron overload might cause diabetic nephropathy. **Materials and Methods:** We performed an association study to assess the role of the C282Y and H63D mutations in the HFE gene as a risk factor for type 2 diabetes and diabetic nephropathy. Altogether, 563 patients with type 2 diabetes were collected. In the analysed group 108 patients had overt proteinuria, 154 microalbuminuria and 301 normoalbuminuria. Among the patients with normoalbuminuria only those with known diabetes duration of at least 10 years were considered as normoalbuminuric (n=162). 196 unrelated healthy subjects were used as a control group. All subjects were genotyped for C282Y and H63D using the PCR-based protocol. **Results:** There was a higher frequency of the 282Y allele carriers among patients with type 2 diabetes than healthy controls (OR 5.3; 95%CI 1.6-17.3). We observed a higher frequency of the 63D allele carriers among patients with diabetic nephropathy (OR 1.8; 95%CI 1.2-2.8). **Conclusion:** In conclusion, our study is the first one which indicates that being a carrier of H63D haemochromatosis mutation is a risk factor for nephropathy in type 2 diabetes. We also confirmed previous observations that the frequency of 282Y mutation was higher in patients with type 2 diabetes than in general population of healthy subjects.

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MECHANICAL STRETCH INDUCES REDUCTION IN ALPHA3 BETA1 INTEGRINS IN GLOMERULAR EPITHELIAL CELLS IN VITRO.

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Background and Aims: Loss of Glomerular Epithelial Cells (GECs) occurs in diabetic kidney disease and is believed to be one of the mechanisms of proteinuria. An early decrease in alpha3 beta1 integrin expression, crucial in the attachment of GECs to the glomerular basement membrane, is seen in animal models of diabetes. We investigated whether alpha3 beta1 expression in GEC is altered by the application of mechanical stretch, mimicking the haemodynamic insult as found in the diabetic glomerulus.

Materials and Methods: Conditionally immortalized murine GEC were grown in DMEM with 5mM glucose at 33 °C with gamma-interferon, and subsequently differentiated at 39 °C for 10 days without gamma-interferon. Mechanical stretch (10% average elongation) was applied using a Flexcell strain unit for 24 and 48 hours. Cells were lysed, subjected to PAGE and immunoblotted with specific antibodies for alpha3 beta1 integrins. Blots were quantitated by densitometry.

Results: After 24 hours stretch, there was no significant effect on alpha3 (stretch vs non-stretch, arbitrary units (au) mean±SD, 1131±628 vs 1082±527) or beta1 integrin (stretch vs non-stretch, 1247±757 vs 1207±730) protein expression levels. However, the application of mechanical stretch for 48 hours induced a 47% reduction in alpha3 integrin (stretch vs non-stretch, 578±195 vs 1143±365, $p<0.02$), and a 38% reduction in beta1 integrin (stretch vs non-stretch, 850±830 vs 1156±731, $p<0.02$) protein expression.

Conclusions: This study shows that GECs are mechano-sensitive and in vitro respond to a mechanical insult by reducing alpha3 and beta1 integrins protein expression. This may represent an important stress-mediated mechanism of GEC loss in diabetic kidney disease.

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THREE POLYMORPHISMS IN THE NEPHRIN GENE ARE NOT ASSOCIATED WITH PROTEINURIA IN TYPE 1 DIABETIC PATIENTS

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BACKGROUND: Congenital nephrotic syndrome (CNF) is characterised by massive proteinuria. Recently, several mutations in the nephrin gene (NPHS1) were found to be responsible for manifest CNF. Three sequence variants were also found in healthy controls. Variations in the NPHS1 gene may affect the degree of proteinuria in patients who do not exhibit the classic clinical course of CNF, which makes this gene an attractive candidate gene for diabetic nephropathy.

OBJECTIVES AND DESIGN: This cross-sectional, case-control study was performed in order to investigate whether these common gene variants are associated with diabetic nephropathy in type 1 diabetic patients.

PATIENTS AND METHODS: We studied 999 C-peptide negative patients from the FinnDiane Study, a comprehensive, multicenter, nation-wide study including all adult type 1 diabetic patients from 17 centres. The mean age was 40±1 yrs, duration of diabetes 28±1 yrs, BMI 25.1±0.1 kg/m², HbA_{1c} 8.5±0.1%. The patients were classified based on their AER: normoalbuminuria with a duration of > 15 yrs (NA, n=323), microalbuminuria (MI, n=170), proteinuria (MA, n=322), ESRD (n=184).

RESULTS: The frequencies of the mutant alleles in the Glu117→Lys, Arg408→Gln and Asn1077→Ser polymorphisms in the entire cohort were 34%, 8% and 12%, respectively. The genotypes did not differ between any of the groups (table).

Genotype (mutants)	NA	MI	MA	ESRD
Glu/Lys + Lys/Lys n (%)	174 (54.4)	92 (54.4)	172 (55.1)	98 (60.1)
Arg/Gln + Gln/Gln n (%)	49 (15.2)	27 (15.9)	56 (17.4)	27 (14.8)
Asn/Ser + Ser/Ser n (%)	78 (24.3)	39 (23.0)	68 (21.3)	39 (21.3)

Further subdivision of the groups into quartiles based on duration of diabetes, AER, HbA_{1c} and serum creatinine showed no significant differences between the groups.

CONCLUSION: This study does not support an involvement of the three polymorphisms of the nephrin gene in the pathogenesis of diabetic nephropathy in type 1 diabetic patients.

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Podocyte Structure and Renal Function in Caucasian Type 2 Diabetic Patients
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Background and Aims: Podocyte loss and structural changes have been suggested to be related to progression of nephropathy in type 2 diabetic (D2) Pima Indians. We studied podocyte number and structure to define the relationships with albumin excretion rate (AER) and glomerular filtration rate (GFR) in Caucasian D2. **Materials and Methods:** Kidney biopsies were performed in 44 D2: 13 were normoalbuminuric-NA, 15 microalbuminuric-MA, 16 proteinuric-P. GFR was measured by plasma clearance of ⁵¹Cr-EDTA. Morphometric analysis was used to estimate: numerical density of podocytes per glomerulus [Nv(podn/glom)-µm³×10⁹], filtration slit density [FSLv-µm/µm³], foot process width (FPW-nm), mesangial fractional volume [Vv(mes/glom)] and glomerular basement membrane (GBM) width (nm). **Results:** HbA_{1c} was different among groups (7.4±1.5% in NA, 8.8±1.5 in MA, 8.8±1.8 in P, Anova <0.05) as was GFR (102±20 ml/min/1.73m² in NA, 112±32 in MA, 83±30 in P, Anova<0.03).

	NA	MA	P
Nv(podn/glom)	160±80*	110±50	80±40
FSLv	0.30±0.08**	0.22±0.06	0.18±0.07
FPW	535±95#	673±117	656±170
Vv(mes/glom)	0.22±0.03	0.26±0.06	0.36±0.09##
GBM width	394±54	488±102	596±142##

Mean±SD. *Anova<0.003, p<0.05 vs MA and P; **Anova<0.001, p<0.05 vs MA and P; #Anova <0.03, p<0.05 vs MA; ##Anova<0.001, p<0.05 vs NA and MA.

AER was inversely related to Nv(podn/glom) (r=-0.54, p<0.001) and FSLv (r=-0.63, p<0.0001) and directly to FPW (r=0.45, p<0.005). GFR was directly related to FSLv (r=0.42, p<0.01) and inversely to FPW (r=-0.40, p<0.05). AER was also directly related to Vv(mes/glom) (r=0.67, p<0.0001) and GBM width (r=0.67, p<0.0001); an inverse correlation was also found between GFR and Vv(mes/glom) (r=-0.67, p<0.0001) and GBM width (r=-0.48, p<0.001). Since 6 pts with abnormal AER had normal Vv(mes/glom) we compared their podocyte structure to that of 12 NA and normal Vv(mes/glom): Nv(podn/glom): 98±50 vs 160±80, p<0.05; FSLv: 0.24±0.04 vs 0.30±0.08, p=0.06). **Conclusions:** changes in podocyte structure and number occur in the early stages of diabetic nephropathy and contribute along with mesangial expansion and GBM thickening to determine renal dysfunction in D2.

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GLUT1 IS UPREGULATED IN GLOMERULAR HYPERTENSION: POSSIBLE ROLE IN THE PATHOPHYSIOLOGY OF DIABETIC GLOMERULOPATHY.

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Background and Aims: Metabolic and haemodynamic insults are important determinants of extracellular matrix deposition by mesangial cells. GLUT-1 is implicated in glucose-induced tissue injury and is upregulated by mechanical stretch in human mesangial cells in vitro. We tested whether high intraglomerular pressure, in the absence of hyperglycaemia, determines upregulation of the GLUT-1 glucose transporter in the glomeruli in vivo.

Materials and Methods: The expression of GLUT-1 was determined in glomeruli of Dahl Salt-sensitive (DS) rats on a high salt diet (NaCl: 4%)(DSH)(a model of systemic and intraglomerular hypertension, Systolic Blood Pressure -SBP- 235±15 mmHG). Spontaneously Hypertensive Rats (SHR)(a model of systemic hypertension without intraglomerular hypertension, SBP 230±10 mmHG), DS on low salt diet (NaCl: 0.5%)(DSL)(SBP 147±8 mmHG), and Wistar Kyoto (WKY) normotensive control (SBP 137±3 mmHG) rats. Snap frozen kidney specimens from 10-12 week-old male DSH (n=3), SHR (n=3), DSL (n=4), and WKY (n=3) rats were studied. Coded kidney specimens were cut in 4-µm section, fixed and stained for immunohistochemistry using a specific GLUT-1 antibody. Four independent, masked, observers scored semiquantitatively the GLUT-1 expression levels in the kidney specimens.

Results: Intense positive staining for GLUT-1 was observed in the glomeruli of the DSH rat group only (DSH: +++ vs DSL: +/-, arbitrary units). The glomeruli of SHR and WKY rats showed virtually no staining for GLUT-1, comparable to that of the DSL rats. In all groups positive staining for GLUT-1 was found in the tubuli.

Conclusions: Glomerular, but not systemic, hypertension upregulates GLUT-1 expression in the glomeruli. We suggest that glomerular hypertension contributes to the pathology of diabetic glomerulopathy by inducing GLUT-1 transporters, thus potentiating the deleterious effect of hyperglycaemia.

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HIGH GLUCOSE IS NECESSARY FOR HEXOSAMINE-INDUCED TGFβ1 EXPRESSION IN HUMAN MESANGIAL CELLS.

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Background and Aims: The mechanisms of glucose-induced TGFβ1 in the kidney remain to be fully elucidated. We determined whether the hexosamine biosynthetic pathway (HBP) is involved in TGFβ1 mRNA expression in human mesangial cells (HMC). We increased the cellular content of hexosamine end-products (a) by culturing HMC in the presence of glucosamine and (b) by over-expression of Glutamine: Fructose-6-phosphate Aminotransferase (GFAT), the rate-limiting enzyme of HBP. We inhibited the HBP by use of azaserine, a competitive inhibitor of GFAT.

Materials and Methods: HMC were transfected with a recombinant defective adenoviral vector expressing GFAT or β galactosidase (β-gal)(control vector) and studied in normal glucose (NG)(6 mM), high glucose (HG)(25 mM), and HG with azaserine (10 µM). The HBP product UDP-N-acetylglucosamine (UDP-NAG) was determined by capillary electrophoresis. TGFβ1 mRNA levels were determined by competitive RT-PCR and expressed as TGFβ1/β-Actin mRNA ratio.

Results: In GFAT transfected cells, but not in β-gal, HG determined a significant 1.9 fold (p<0.024) increase in UDP-NAG compared to NG. HG increased TGFβ1 mRNA levels by 2 fold (p<0.039) versus NG in β-gal transfected cells; azaserine attenuated this effect. In HG, but not in NG, GFAT transfected cells showed a 2.5 fold increase in TGFβ1 expression versus β-gal transfected cells, an effect completely inhibited by azaserine. Addition of glucosamine (12 mM) resulted in cell death.

Conclusions: Overexpression of GFAT in HG greatly increases TGFβ1 mRNA expression. However, in NG, GFAT overexpression is not sufficient to increase TGFβ1 mRNA expression, possibly because of insufficient substrate availability.

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Insulin Signalling Pathways

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Transfection of the G972R variant of the IRS-1 gene in 3T3L1 adipocytes impairs the insulin signalling pathway and inhibits cell differentiation.

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Background and Aims: The molecular mechanisms responsible for insulin-resistance are still unknown. The Insulin Receptor Substrate-1 (IRS-1) plays a central role in insulin sensitivity and defects in the IRS-1 protein may be involved in the development of insulin-resistance. Association studies have shown that the IRS-1 gene G972R variant is a genetic risk factor for insulin-resistance. However, how this mutation may lead to impaired insulin sensitivity is still to be determined. Aim of our study is to evaluate, after transfection of the IRS-1 G972R variant in 3T3L1 adipocytes, the effects of this mutation on insulin signalling and on cell differentiation.

Materials and Methods: The 3T3L1 cells were transfected with pcDNA3 expression vector containing either the human wild-type IRS-1, the G972R variant or the pcDNA3 vector alone. After incubation with selective medium, individual geneticin G418-resistant stable cell clones were selected for the study.

Results: Western blotting revealed the expression of transfected IRS-1 in 3T3L1 cells. After the induction of differentiation the 3T3L1 transfected cells with wild-type IRS-1 and with pcDNA3 alone differentiated in 6-8 days, as expected. Surprisingly, the cells transfected with the G972R variant did not differentiate. To determine whether the defect in IRS-1 was responsible for this alteration, we firstly analysed by Northern blot the expression of PPAR-gamma gene, which plays a central role in the insulin signalling cascade that stimulates adipocyte differentiation. The PPAR-gamma mRNA was absent in the cells transfected with the mutated IRS-1. Also AdipoQ and adipisin, both markers of adipocyte differentiation, were absent. To establish which proteins upstream of PPAR-gamma may be involved, we studied by the presence of several intracellular proteins, including MAP kinases and AKT. Results showed that P38 and P42/44 MAPK (involved in cell proliferation) remained phosphorylated until the 8th day of differentiation in G972R cells, whilst MAPK phosphorylation in wild-type IRS-1 cells decreased after the 2nd day. Also AKT resulted highly phosphorylated in G972R cells, probably secondary to high levels of P38 MAPK. Proteins downstream of AKT, like ADD1 and C/EBP-alpha are currently under study.

Conclusions: The G972R variant of the IRS-1 gene appears to impair the insulin signalling pathway of 3T3L1 adipocytes, inhibiting the differentiation of these cells, possibly through an alteration of the cascade involving the transcription of the PPAR-gamma gene.

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GLYCOGEN SYNTHASE KINASE-3 AND AMP-ACTIVATED PROTEIN KINASE ACTIVITY IN SKELETAL MUSCLE FROM TYPE 2 DIABETIC SUBJECTS

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Background: A defect in insulin-stimulated glycogen synthase (GS) activity in skeletal muscle is a consistent finding in type 2 diabetes. GS is activated by insulin, mainly through dephosphorylation of specific serine residues designated site 2, 3a and 3b. GSK3 phosphorylates site 3a and 3b in vivo and has been shown to be inhibited by insulin. AMPK phosphorylates site 2 in vitro and has recently been shown to co-immunoprecipitate with GS in vivo.

Aims and methods: To investigate the potential role of GSK3 and AMPK in reduced GS activity in type 2 diabetes, the activity of GS, GSK3α/1-AMPK and α2-AMPK were studied in biopsies of m. vastus lateralis from 10 type 2 diabetic and 10 matched control subjects before and after 4-h euglycemic-hyperinsulinemic clamp (40 mU/m²/min).

Results: Insulin-stimulated glucose disposal was significantly decreased in type 2 diabetic subjects (177±26 vs 290±25 mg/m²/min, P < 0.01) mainly due to a decreased rate of insulin mediated non-oxidative glucose metabolism (113±20 vs 196±21 mg/m²/min, P = 0.02). Insulin-stimulated GS activity was significantly reduced in type 2 diabetic subjects (fractional velocity: 25±2 vs 40±4%, P < 0.01). Insulin decreased GSK3α activity equally in both groups; from 3.9±0.2 to 2.6±0.2 nmol/mg/min in diabetic subjects (P < 0.01) and from 4.1±0.4 to 2.6±0.1 nmol/mg/min in control subjects (P < 0.01), whereas α1-AMPK and α2-AMPK activities were equally unaffected by insulin in the two groups.

Conclusion: We conclude that the defect in insulin-stimulated glycogen synthase (GS) activity in skeletal muscle from type 2 diabetic patients may not be caused by changes in GSK3 or AMPK activity.

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THE ROLE OF ATYPICAL AND CONVENTIONAL PKC IN DEHYDROEPIANDROSTERONE-INDUCED GLUCOSE UPTAKE

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Background and Aims: We have reported that both dehydroepiandrosterone (DHEA) and dexamethasone (Dexa) directly activate PKC. In this study, we investigated the effects of these hormones on conventional PKC (cPKC) and atypical PKC (aPKC). **Results:** DHEA and Dexa directly activated PKCβ and PKCζ to the same degree. In rat adipocytes, DHEA and Dexa activated endogenous immunoprecipitable PKCζ to 246% and 164%, respectively from basal level (100%). In adipocytes, 5 min treatment with DHEA increased phosphatidylinositol 3-kinase (PI 3-kinase) activity in immunoprecipitate with anti-phosphotyrosine antibody to 235%. Preincubation with wortmannin, myristoylated PKCζ pseudosubstrate, but not with Go6976, abolished DHEA-induced 2-deoxyglucose (DOG) uptake. cPKC inhibitors prevented Dexa-induced insulin resistance. Moreover, DHEA and Dexa increased DOG uptake to 330% and 220%, respectively, in adipocytes overexpressed with wild-type PKCζ, but not in those overexpressed with dominant negative. **Conclusions:** These results indicated that DHEA and Dexa activate both cPKC and aPKC, and Dexa-induced cPKC activation may lead to insulin resistance. In contrast, DHEA may mimic or enhance insulin action via PI 3-kinase and aPKC.

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Antisense Protein Tyrosine Phosphatase 1B Lowers Plasma Glucose, Increases Insulin Sensitivity and Reverses Activation of the Stress Kinase p38 in ob/ob Mice. R.J. Gum, B. Zinker, C. Rondinone, J. Clampit, J. Waring, L. Gaede, M. Stark, M. Heindel, N. Xie, J. Trevillyan, M. Jirousek and R. Ulrich. Abbott Laboratories, Abbott Park, IL USA.

Background and Aims: This study was designed to determine if inhibiting PTP1B (protein tyrosine phosphatase 1B), using antisense technology, lowers plasma glucose and increases insulin sensitivity in a diabetic animal model, without toxic side effects, and to identify signaling mechanisms involved. **Materials and Methods:** ob/ob mice (6-7 wks) were treated with antisense PTP1B (ISIS-113715) for 6 weeks at 25 mg/kg, 2X/wk. Glucose and insulin levels were determined weekly. A subset of mice was stimulated with insulin i.p. for 1 or 5 min. Western blotting and RNA analysis including arrays were performed on liver and fat for various molecules to identify signaling pathways affected. **Results:** Antisense PTP1B normalized plasma glucose (p<0.01) in diabetic ob/ob mice to lean ob/+ levels and produced evidence of increased insulin sensitivity in these mice without causing hypoglycemia and without overt toxicity at efficacious doses. Insulin signaling pathways were activated in liver and fat of antisense PTP1B treated ob/ob mice including insulin receptor substrate 2 protein (IRS-2, 2-fold in liver and 4-fold in fat (p<0.05 and 0.001, respectively) and protein kinase B activity (PKB, 2 fold in liver, p<0.05). Downstream gene expression in the gluconeogenesis pathway, including phosphoenolpyruvate carboxykinase (PEPCK), was inhibited in liver. Insulin receptor (IR) phosphorylation and PKB phosphorylation was enhanced (3 and 6-fold, respectively, p<0.01, each) in liver of antisense treated mice stimulated with insulin for 1 or 5 minutes. By contrast activation of extracellular signal-regulated kinase (ERK) mitogen activated protein kinase (MAPK) was not enhanced upon insulin stimulation in either tissue. In addition, activation of p38 MAPK was decreased by 70% (p<0.001) in antisense PTP1B treated ob/ob mice to lean ob/+ levels. **Conclusions:** Decreasing PTP1B mRNA and protein, through antisense oligonucleotides, normalizes plasma glucose and insulin levels in diabetic animals. Decreasing protein levels by 50% is sufficient for effective treatment. Downstream elements in the insulin signaling pathways are activated particularly in response to insulin. Activation of the stress kinase p38 is reversed indicating a decrease in activation of stress pathways upon PTP1B inhibition and glucose normalization. This provides evidence that similar decreases in PTP1B protein in humans may be an effective treatment for type 2 diabetes.

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ROLE OF DIACYLGLYCERIDES AND CERAMIDES IN THE EFFECTS OF PALMITATE ON GLUCOSE UPTAKE IN CULTURED HUMAN MUSCLE
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Background and Aims: The mechanisms that trigger muscle insulin resistance in type 2 diabetes are still unclear; however, a strong correlation between insulin desensitization and intramuscular lipid accumulation has been found. Several second messengers deriving from lipid metabolism have been proposed to underlay this effect. We have previously shown that accumulation of saturated NEFA impaired insulin-stimulated glucose uptake, whereas unsaturated NEFA had no effect. Here we examine the involvement of diacylglycerides (DAG) and ceramides on the effects of saturated NEFA on glucose metabolism and their relation to the lipid-dependent protein kinases involved in insulin signaling. **Materials and Methods:** This study was performed in human muscle primary culture. DAG and ceramide levels were determined by thin-layer chromatographic analysis. **Results:** Muscle cells were preincubated for 15 h in 25 mmol/l glucose-DMEM in the absence or presence of 0.25 mmol/l palmitate. This treatment increased both DAG and ceramide levels by about 3-fold. Basal glucose uptake was increased and insulin-stimulation was inhibited in palmitate-incubated cells. The level of Thr-308 phosphorylation of protein kinase (PK) B was elevated by insulin, irrespective of palmitate treatment. However, palmitate did not modify basal PKB phosphorylation, whereas it increased PKC activity in plasma membranes. Treatment of cells with GF 109203X, a selective inhibitor of DAG-sensitive PKCs, did not modify basal glucose uptake nor did it alter the effect of palmitate, whereas it enhanced the stimulation by insulin. **Conclusions:** Although palmitate promotes the accumulation of ceramides in human skeletal muscle, our data indicate that it does not alter basal or insulin-stimulated PKB phosphorylation. Rather, the effects of palmitate appear to be mediated through the activation of DAG-insensitive PKCs.

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Effect of 4-hydroxyisoleucine on insulin sensitivity in insulin resistant rats.

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4-hydroxyisoleucine (4-OH-Ile), an amino acid extracted from fenugreek seeds, stimulates insulin secretion only in the presence of a stimulative glucose concentration. It induces a decrease in blood glucose and insulin concentration in type 2 diabetic rats, suggesting a possible effect on insulin sensitivity. The aim of this work was therefore to determine the effect of 4-OH-Ile on insulin sensitivity in normal and insulin resistant rats. **Animals and methods:** male Sprague-Dawley rats were submitted for 3 weeks to a control, or a saccharose (57.5%) and lipid (14%), diet and subsequently investigated during sequential euglycemic hyperinsulinemic (basal, followed by insulin infusion rate 0.4U/kg/h) clamp performed in the absence, or in the presence, of a 20 mg/kg/h 4-OH-Ile infusion. **Results:** 1) the saccharose-lipid diet induced insulin resistance demonstrated by the decrease in the glucose infusion rate R'a observed during insulin infusion (7.06±1.48 vs 11.78±0.82 mg/kg/min, saccharose-lipid vs control diet, p=0.0143). 2) In control rats (n=8), insulin infusion produced a decrease in glucose production rate Ra (6.42±1.3 vs 11.41±0.78 mg/kg/min p=0.0099), insulin vs basal, which was blunted in saccharose-lipid fed rats (n=8), (8.54±1.43 vs 10.68±1.42 mg/kg/min p=0.2847). Insulin induced an increase in glucose utilization, Rd (18.3±1.3 vs 11.41±0.78 mg/kg/min p=0.005, insulin vs basal). This effect was blunted in saccharose-lipid fed rats (15.60±0.87 vs 10.68±1.42 mg/kg/min p=0.0175). 3) The infusion of 4-OH-Ile corrected completely insulin resistance in the saccharose-lipid fed rats (R'a=10.99±1.07 mg/kg/min, p=0.0487 vs absence of 4-OH-Ile). 4-OH-Ile produced a significant increase in Rd (22.14±1.18 and 18.6±1.8 mg/kg/h in control and saccharose-lipid fed rats, respectively, p=0.0135 vs absence of 4-OH-Ile). The molecule had no effect on glucose production. **Conclusion:** In addition to its stimulatory effect on insulin secretion, the 4-OH-Ile improves insulin sensitivity in this experimental model.

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INCREASED INSULIN SENSITIVITY IN IGF-1 RECEPTOR-DEFICIENT BROWN ADIPOCYTES.

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Background and Aims: Insulin like growth factor I (IGF-I) is a mitogen also involved in adipogenic- and thermogenic-differentiation in fetal brown adipocytes. We have characterized immortalized cell lines derived from the brown adipose tissue of fetuses of mice deficient in the IGF-1 receptor, as well as the study of insulin action in these cells.

Materials and Methods: Generation of cell lines with SV40 LTag, Immunoprecipitation, Western and Northern blot, Phosphatase activity and DNA synthesis experiments were performed.

Results: Brown adipocyte cell lines from fetuses of mice deficient in the IGF-IR gene maintained the expression of adipogenic- and thermogenic-differentiation markers and showed a multilocular fat droplets. Insulin-induced autophosphorylation of the insulin receptor b-chain was augmented in IGF-IR-/- cells as compared to the wild type. Insulin-induced tyrosine phosphorylation of IRS-1 was higher in IGF-IR-/- brown adipocytes despite that its protein content was lower than that of wild type cells. In contrast, tyrosine phosphorylation of IRS-2 decreased in IGF-IR-deficient cells, its protein content being unchanged as compared to the wild type. Downstream IRSs, the association IRS-1/Grb-2 was augmented in IGF-IR-/- cells. However, no differences in SHC tyrosine phosphorylation and its association with Grb-2 in response to insulin were observed. IGF-IR-/- cells showed an enhanced activation of the mitogen-activated protein kinase (MAPK) cascade upon insulin stimulation. In addition, the lack of IGF-IR resulted in a higher mitogenic response to insulin as compared to wild type cells. Finally, cells lacking IGF-IR showed a much lower association between IR or IRS-1 and phosphotyrosine phosphatase 1B (PTP1B) and also a decreased PTP1B activity upon insulin stimulation.

Conclusions: IGF-IR-deficient brown adipocytes show an increased insulin sensitivity via IRS-1/Grb-2/MAPK, resulting in increased mitogenesis.

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Intramyocellular lipid is associated with in vivo insulin resistance on glucose uptake and insulin signalling defects in human skeletal muscle.

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Background and Aims. To examine whether and how intramyocellular lipid (IMCL) content contributes to interindividual variation in insulin action, we studied 20 healthy men with no family history of type 2 diabetes. IMCL was measured using proton magnetic resonance spectroscopy in vastus lateralis muscle and normalized for muscle total creatinine (CrT).

Materials and Methods. Whole body insulin sensitivity was measured using a 120-minute euglycemic hyperinsulinaemic (insulin infusion rate 40 mU/m² min) clamp. Muscle biopsies of vastus lateralis muscle were taken before and 30 min after initiation of the insulin infusion to assess insulin signalling. The subjects were divided into groups with high (9.5±0.9 IMCL/CrT, n=10, HiIMCL) and low IMCL (3.0±0.5 IMCL/CrT, n=10, LoIMCL), the cutpoint being median IMCL (6.1 IMCL/CrT).

Results. The groups were comparable with respect to age (43±3 vs. 40±3 yrs, NS, HiIMCL vs. LoIMCL), body mass index (26±1 vs. 26±1 kg/m², NS), and maximal oxygen consumption (33±2 vs. 36±3 ml/kg min, NS). Whole body insulin stimulated glucose uptake was lower in the HiIMCL (3.0±0.4 mg/kg min) than the LoIMCL (5.1±0.5 mg/kg min, p<0.05) group. Study of insulin signalling indicated that insulin induced tyrosine phosphorylation of the insulin receptor (IR) was blunted in HiIMCL compared to LoIMCL (57% vs. 142% above basal, p<0.05), while protein expression of the IR was unaltered. Insulin receptor substrate-1 associated phosphatidylinositol-3-kinase activation by insulin was also lower in HiIMCL than in LoIMCL (49±23 vs. 84±27 % above basal, p<0.05 between HiIMCL and LoIMCL).

Conclusions. IMCL accumulation is associated with whole body insulin resistance and with defective insulin signalling in skeletal muscle independent of body weight and physical fitness.

PS 1

Type I Diabetes – Genetics 1

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MORTALIN – A PUTATIVE CANDIDATE GENE FOR IDDM?

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Background and Aims: Mortalin has by 2D-protein gel analysis been found to be upregulated in isolated rodent islets exposed to cytokines. As mortalin furthermore has been associated with cellular senescence, we consider the gene encoding for mortalin (Sq31.1) a putative candidate gene for cytokine induced beta-cell destruction. **Materials and Methods:** A population based Danish IDDM family material comprising 257 families. Single stranded conformation polymorphism screening of the human mortalin gene expressed in cytokine stimulated leukocytes (mortalin cDNA). Genotyping typing: (i) a RFLP assay (enzyme: Ddel), and (ii) a PCR based microsatellite assay for the D5S500 dinucleotide marker. Linkage testing: transmission disequilibrium tests. **Results:** Three novel polymorphisms at the cDNA level in position 272, 977 and 1962 (Genbank: L15189) were identified in the mortalin gene. When establishing genotyping assays for the identified cDNA polymorphisms in genomic DNA we identified mismatches between the cDNA and genomic DNA sequences. The recent release of a human BAC-clone comprising genomic DNA from chr. 2, showed 100% homology to the genomic DNA sequence we obtained, suggesting the existence of a mortalin pseudogene on chr. 2. Despite having this sequence information it was only possible to establish a RFLP genotyping assay for the A to G polymorphism in position 977. This polymorphism was not found to be in linkage in a Danish IDDM population. Furthermore, we tested the polymorphic D5S500 dinucleotide marker located less than 2.3 cM from the mortalin gene at Sq31.1 without finding linkage to IDDM. **In conclusion,** the mortalin gene is unlikely to be an IDDM candidate gene, as linkage was neither established for one of the three novel identified polymorphisms within the coding sequence of the gene nor for a microsatellite located close to the mortalin in a Danish IDDM family material. We suggest the existence of a mortalin pseudogene on chr. 2.

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A NOVEL POLYMORPHISM IN THE PROMOTER REGION OF THE IL-4 GENE IS ASSOCIATED WITH TYPE I DIABETES IN JAPANESE

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Background and Aims: IL-4, one of the key regulators of the Th1-Th2 balance, promotes the differentiation of CD4+ helper lymphocytes into Th2 cells. It is assumed that the Th1 predominance over Th2 has an important role in the development of type 1 diabetes. In the present study, therefore, we investigated the possible role of the IL-4 gene in type 1 diabetes. **Materials and Methods:** We studied 241 type 1 diabetic patients and 369 control subjects. First, we screened for variations in the promoter region (position -1105 to +38; +1= translation start site) and exons of the IL-4 gene in 24 patients by PCR direct sequencing. Next, genotyping of each polymorphism was performed by PCR-RFLP. **Results:** PCR direct sequencing detected five polymorphisms; T(-590)C, C(-144)T, T(-33)C, A(+363)T, and A(+841)C. Among them, A(+363)T and A(+841)C are located in introns and were not further analyzed. C(-144)T was a novel and relatively rare polymorphism, and we found that the distribution of C(-144)T genotype was significantly different between type 1 diabetic patients and control subjects. CT genotype was observed in eight type 1 diabetic patients (3.3%) and three controls (0.8%), and the remainder were all CC genotype (odds ratio of CT genotype: 4.2, $p=0.030$). No significant difference was observed in the distribution of T(-590)C and T(-33)C genotypes between patients and controls. We also found that T(-590)C and T(-33)C were in significant linkage disequilibrium, but C(-144)T was not significantly associated with both T(-590)C and T(-33)C. **Conclusions:** We provided evidence for the first time that IL-4 gene polymorphism contribute to the development of human type 1 diabetes. Further genetic and functional studies, however, are required.

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SIBPAIR MAPPING OF SUSCEPTIBILITY LOCI TO TYPE 1 DIABETES IN RUSSIAN FAMILIES

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Background and Aims: In this study we have performed the sibpair analyses to map genes that predispose to type 1 diabetes using 35 affected sibpair and 66 simplex families of Russian origin. **Materials and Methods:** Polymorphic markers were amplified using PCR and primer sequences from GenBank. To identify alleles PCR products were separated in 12% polyacrylamide gel and stained with silver nitrate. Combined transmission/disequilibrium test (TDT) and sib TDT (S-TDT) were used for data analysis. **Results:** We have studied linkage of several sets of polymorphic markers located at regions containing *IDDM3*, *IDDM9*, *IDDM10* and *IDDM12* susceptibility loci. In case of *IDDM3* locus (6q25.3-q27) linkage evidences have been obtained for marker *Ala(-9)Val* ($z'=2.25$, $p<0.05$; $MLS=2.06$, $p<0.05$) located at mitochondrial superoxide dismutase gene (*SOD2*) but not for marker *D6S503*. In *IDDM9* locus (3q21-q25) we have found linkage evidences for two microsatellites: *D3S1769* ($z'=1.68$, $p=0.04$) and *D3S4015* ($z'=1.809$, $p=0.03$). In case of *IDDM10* locus (10p11.2) linkage evidences have been obtained for markers *D10S1243* ($z'=3.83$, $p<0.0001$) and *D10S2326* ($z'=2.066$, $p=0.02$) but not for markers *D10S1426* and *D10S507*. In case of *IDDM12* locus (2q33) two polymorphic markers of the human cytotoxic T lymphocyte associated antigen-4 gene (*CTLA-4*), namely, codon 17 dimorphism and (AT)_n microsatellite located in the 3'-untranslated region have been developed and the linkage of these markers with diabetes type 1 have been studied. A nonrandom excess of the Ala17 CTLA4 molecular variant ($MLS=3.26$, $p=0.000015$; $z'=4.07$, $p=0.00003$) and the 104-bp allele of the dinucleotide marker ($MLS=3.14$, $p<0.000001$; $z'=3.96$, $p=0.0007$) have been observed in affected and simplex sibling pairs, correspondingly. **Conclusions:** Using families of Russian origin we have found evidences of linkage for all studied loci (*IDDM3*, *IDDM9*, *IDDM10* and *IDDM12*) previously identified as potential susceptibility loci in British and USA populations. The several markers located in these loci are strongly associated with, and linked to diabetes type 1 in a Russian population.

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CTLA-4 G allele is associated with Autoimmune Polyglandular Syndrome type II in patients negative for susceptible haplotypes HLA DRB1*03-DQB1*0201 and DRB1*04-DQB1*0302

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Background and Aims: The Autoimmune Polyglandular Syndrome Type II (APS II) is characterized by Addison's disease occurring concomitantly with Type 1 diabetes and an autoimmune thyroid disease. APS II is strongly associated with HLA DR and DQ genes and is more commonly present in females. The aim of our study was to analyze the HLA DR and DQ polymorphism and the CTLA-4 exon 1 A49G dimorphism and their possible interactions in the susceptibility to APS II. **Materials and Methods:** We have typed DRB1 and DQB1 loci using a PCR/reverse lineblot method, and the CTLA-4 exon 1 position A49G dimorphism using a PCR-RFLP method from 41 APS II patients and 302 matched controls from Italian population.

Results: The HLA DRB1*03-DQB1*0201 and DRB1*04-DQB1*0302 haplotypes are associated with APS II ($p<10^{-4}$; OR 4.17 and 4.6, respectively). The DRB1*03-DQB1*0201/DRB1*04-DQB1*0302 genotype is statistically increased in APS II patients compared to control subjects (15% vs 2%, respectively, $p<10^{-4}$) and confers the highest risk (OR=10.7). CTLA-4 G allele was found associated with APS II only in patients negative for HLA DRB1*03-DQB1*0201 and DRB1*04-DQB1*0302 ($p=0.003$).

Conclusions: CTLA-4 confers risk only in patients negative for HLA DRB1*03-DQB1*0201 and DRB1*04-DQB1*0302 haplotype. We found association of HLA DR and DQ with APS II also in Italian population suggesting that this region plays the major role in the susceptibility to the disease.

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NO MAJOR ROLE OF THE 3' UTR POLYMORPHISM OF THE IL-12P40 GENE (IL12B) IN TYPE 1 DIABETES AND AUTOIMMUNE THYROID DISEASE IN JAPANESE

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Background and Aims: IL-12 promotes the differentiation of CD4⁺ helper lymphocytes into Th1 cells, which are essential in the development of type 1 diabetes. Very recently, new type 1 diabetes susceptibility locus, *IDDM18*, has been mapped near IL-12p40 gene (*IL12B*), and significant bias in transmission of the *IL12B* 3' UTR single nucleotide polymorphism (SNP) to type 1 diabetic patients was observed. In the present study, therefore, we investigated the possible role of the 3'UTR SNP of *IL12B* in type 1 diabetes (T1D) and autoimmune thyroid disease (AITD) in Japanese. **Materials and Methods:** We studied 185 T1D patients, 91 patients with Graves' disease (GD) and 57 patients with Hashimoto's thyroiditis (HT) and 395 control subjects. Genotyping of the was performed by PCR-RFLP using endonuclease TaqI. In the Caucasian population, A allele (TaqI negative) was reported to be predominant (frequency ~0.8) and susceptible to T1D. **Results:** In the control subjects, allele frequencies were 0.465 for A and 0.535 for C. In the patient groups, frequencies of A allele were 0.511 (T1D), 0.456 (GD) and 0.526 (HT). Thus, A allele was increased in the T1D patients and the HT patients compared to the control subjects, but the differences were both insignificant ($p=0.15$, $p=0.23$, respectively). Also, we found no significant difference in the genotype distribution between groups. Furthermore, stratification of T1D patients by HLA genotypes and age at onset did not yield significant association. **Conclusions:** In Japanese, the 3'UTR SNP of *IL12B* is also polymorphic but may not play an important role in the pathogenesis of T1D and AITD.

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GENETIC PREDISPOSITION FOR TYPE 1 DIABETES AMONG UNAFFECTED FIRST-DEGREE RELATIVES OF DIABETIC PATIENTS.

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Background and Aims. Type 1 diabetes mellitus (T1DM) is a common autoimmune disease appearing in genetically predisposed individuals. However, the maximum reported concordance rate of T1DM in monozygotic twins (30-50%) indicates the importance of environmental factors. In order to assess indirectly the importance of environmental factors, we analysed the presence/absence of susceptibility genes previously reported as associated with T1DM in the Romanian population (IDDM1, IDDM2 and ICAM1) in a group of non-diabetic first degree relatives of T1DM patients. **Materials and Methods.** The study group comprised 544 unaffected parents, brothers and sisters (265 M/279 F) of 212 type 1 diabetic patients. The typing for IDDM1, IDDM2 and ICAM1 was made using the SSP-PCR method. We define as HLA(+) those patients DR3 or DR4 positive and as HLA(-) patients those DR3 and DR4 negative. We define as INS(+) those patients homozygotes for Class II Ins-VNTR alleles and as INS(-) those positive for Class III alleles at the same locus. We define as ICAM(+) those patients E/E homozygotes for 469 E/K polymorphism of ICAM1 gene (E allele diabetogenic) and as ICAM(-) those E/K or K/K at the same locus. **Results.** We found 373 HLA(+) subjects (68.6%) vs. 171 HLA(-) subjects (31.4%). INS(+) subjects represented 71.9% (391 of 544) vs. 28.1% INS(-) (153). ICAM(+) subjects were 111 of 544 (20.40%) vs. 79.6% ICAM(-). We found 26 HLA(-)/INS(-)/ICAM(-) subjects (4.78%) vs only 1 (0.47%) of 212 diabetics. Overall 48 non-diabetic subjects (8.82%) carried the most diabetogenic haplotype i.e. HLA(+)/INS(+)/ICAM(+). **Conclusions.** There are extremely rare (1 of 212) the cases of T1DM without genetic susceptibility. There are relatively many cases (48 of 544 i.e. 8.82%) with T1DM genetic susceptibility without developing the disease. So, our data sustain that the genetic susceptibility, though necessary, is not sufficient for T1DM occurrence. This suggests the conjugate intervention of more than one environmental factor.

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HLA-DQ MOLECULES MODIFY THE RISK OF THYROID AUTOIMMUNITY IN TYPE 1 DIABETIC CHILDREN

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Background and Aims: Type 1 diabetes mellitus is frequently associated with thyroid autoimmunity (TAI). Both this diseases share similar risk HLA class II genotypes. The aims of this study were to estimate the prevalence of TAI in diabetic children and to determine the contribution of HLA-DQA1, -DQB1 alleles to TAI susceptibility among diabetic children.

Materials and Methods : 285 diabetic children (158 boys and 127 girls) aged 0 to 18 years were screened for thyroid autoimmunity using autoantibodies against thyroid peroxidase (TPO) and thyroglobulin (Tg). Both TPO and Tg were determined using RIA methods. The HLA-DQA1, -DQB1 genotype was investigated by polymerase chain reaction with a set of 24 sequence-specific primer pairs (PCR-SSP) in all children of the study group. The levels of risk were calculated as odds ratios (OR) and their confidence intervals were expressed according to Woolf's formula.

Results : Repeated positivity of TPO and/or Tg autoantibodies was found in 45/285 diabetic children (15.8%). The prevalence rate was significantly higher in girls than in boys (27% vs 7%, $p<0.0001$). The susceptibility of thyroid autoimmunity in diabetic children was negatively associated with HLA-DQB1*05xx (DQB1*0501, 0502, 0503) conferring the OR 0.20, CI 95% 0.05-0.85, $p<0.03$, and positively associated with HLA-DQB1*0302 (OR 2.6, CI 95% 1.1-6.2, $p<0.04$).

Conclusions : The high prevalence of thyroid autoimmunity found in our group of diabetic children emphasizes the need for their regular screening. We observed the significant protective effect for HLA DQB1*05 and the risk effect for HLA-DQB1*0302 for thyroid autoimmunity within the group of diabetic children.

The study was supported by the Ministry of Health of the Czech Republic (grant no.6278-3) and by the Ministry of Education of the Czech Republic (grant no.111300003).

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POLYMORPHISM IN HLA GENES MARKERS OF TYPE I DIABETES

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Background and aims We studied associations between HLA genes and Type I diabetes susceptibility in 2 ethnic groups: 1) Russian subjects from Moscow (Moscovites); 2) Russian Pomors from North-West of Russia. The choice for Pomors was due to the fact that their Type I diabetes morbidity was revealed 1,8 times higher comparing to Moscovites. **Materials and methods** We used SSP method with original primer kits determining 16 DRB1 specificities, 12 DQA1 alleles, and 20 DQB1 alleles. Besides DRB1*04 allelic variations were determined. Among Type I diabetes' patients the following HLA DRB1 allelic variants were identified: HLA DRB1*0401, *0403, *0404, *0405, *0408. **Results** The data on different haplotypes strongly associated to Type I diabetes are submitted in the table:

N	Haplotypes	Pomors (n=38)		Moscovites (n=79)	
		RR	P	RR	P
1	DRB1*04-DQA1*03-DQB1*0302 DRB1*04-DQA1*0301-DQB1*0302	29.7	<0.001	6.91	<0.05
2	DRB1*04-DQA1*0301-DQB1*0302 DRB1*03-DQA1*0501-DQB1*0201	8.67	<0.001	71.13	<0.001

Different association level between Type I diabetes and DRB1*0401 was revealed too. In Pomors the highest RR (3,44) is determined for DRB1*0404 comparing to RR=2,2 in Moscovites. On the contrary in Moscovites the highest RR (8,8) is determined for DRB1*0401 comparing to 1,25 in Pomors. As for HLA-DRB1*0403 its frequency in "healthy" Moscovites was 2,9% and in Pomors-1,2%. In Type I diabetic Moscovites HLA DRB1*0403 was 1,1%, but was not revealed in Pomors. **Conclusion** In Pomor population with high Type I diabetes morbidity we revealed unusual associations between Type I diabetes and HLA as compared to Moscovites.

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Genetic and pancreatic islet autoimmunity markers in first degree relatives of subjects with type 1 diabetes: the Cuban diabetes prediction and prevention study.

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Background and Aims: In preparing for trials of prevention of type 1 diabetes, we have screened 1065 first-degree relatives (FDRs) of type 1 diabetic subjects (parents, siblings and offspring), from 1-60 years of age (mean 22.6 ± 15.6). The aim of our investigation was to identify FDR with high risk to develop type 1 diabetes, using immunologic, metabolic and genetic markers.

Materials and Methods: All FDRs were examined for fasting blood glucose, islet cell antibodies (ICA) and insulin autoantibodies (IAA). ICA positive FDRs (ICA >10 JDF units in two consecutive determinations) were also investigated for antibodies to glutamic acid decarboxylase (anti-GAD65) and antibodies to tyrosine phosphatase (anti-IA2), intravenous glucose tolerance test (IVGTT) (measuring insulin) and typing of HLA-DR genes. ICAs were determined by indirect immunofluorescence with prolonged incubation. IAAs were detected using a competitive fluid-phase radioimmunoassay. Autoantibodies against isoform 65 KD glutamic acid decarboxylase and IA2 molecule were detected by a quantitative radioimmunoprecipitation assay. HLA Class II typing (DR1 to DR10) was performed on B-cell enriched suspension using a two-step microlymphocytotoxicity assay. Insulin secretion was detected using a radioimmunoassay.

Results: We found ICA in 26 (2.4%) and IAA in 35 (3.3%) FDRs. After 3.5 years of follow-up as mean, 5 out of 26 (19.2%) ICA+ FDRs developed type 1 diabetes whereas only one out of 1039 (0.09%) ICA negative FDRs did ($p < 0.0001$). The latest presented an HLA-DR3/DR4 genotype but was negative for IAA, GAD65 and IA2 antibodies. Before type 1 diabetes onset, new diagnosed type 1 diabetes FDRs had higher frequencies of antibodies against islet cell targets (anti-GAD65, 100% vs 53%, anti-IA2, 60% vs 6%), HLA class II susceptibility genotypes (DR3, 80% vs 57%; DR4, 100% vs 28%) and impaired first phase insulin secretion (IVGTT ($1/3 \text{ min} < 67 \mu\text{U/ml}$), 80% vs 6%) than non-diabetic ICA+ FDRs. So far, 19 ICA+ FDRs attend the Cuban nicotinamide prevention trial.

Conclusions: Our results confirm that ICA determination is the best predicting marker for the development of type 1 diabetes which combined with other metabolic, immunological and genetic tests may improve the identification of pre-diabetic subjects who require a prevention trial.

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PREVALENCE AND RISK OF HLA-DRB1 AND -DQB1 GENOTYPES IN AUTOANTIBODY POSITIVE HEALTHY SCHOOLCHILDREN

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Background and Aims: In recent onset type 1 diabetic patients and autoantibody (AAb) positive relatives, a concordance between HLA markers and humoral markers of the disease susceptibility has been observed. This study was aimed to compare HLA-DRB1 and DQB1 specificities of AAb positive and AAb negative healthy schoolchildren with type 1 diabetic patients. **Materials and Methods:** For this, 203 AAb positive probands representing a cohort of 6,155 schoolchildren aged 6-17 years, 339 age- and sex-matched AAb negative schoolchildren and 274 type 1 diabetic patients were involved. AABs against GAD (GADA), protein tyrosin phosphatase (IA-2A), insulin (IAA) ≥ 99 centile were determined by radioassays and islet cell antigens (ICA) ≥ 20 JDF units immunohistochemically, the HLA DQB1 and DRB1 alleles were analyzed by DNA typing. **Results:** Among AAB positive children, GADA were present in 93 (45.8%), IA-2A in 57 (28.3%), IAA in 53 (26.1%) and ICA in 60 (29.5%) children. Co-occurrence of different AAB specificities was seen in 36 (17.7%) probands. GADA were highest among DRB1*04/*04 and DQB1*0302/*0302 homozygotes (87.5% and 83.3%, respectively). ICA ≥ 20 JDF units were preferentially seen in DRB1*03/*03 homozygotes (8/10). Stratification by AAB specificity and subsequent comparison with AAB negative controls for the prevalence of associated and protective HLA markers indicated significant positive (odds ratio range 1.9-16.9) and negative (range 0-0.5) associations. Negative association was most marked in GADA and particularly IA-2A positive children and in probands with multiple AABs. In comparison to diabetic patients, the HLA associations in AAB positive schoolchildren were considerably lower, suggesting that only a minor part of AAB positive children will progress to clinical diabetes. **Conclusions:** Normal population-based screening strategies to identify subjects at risk should combine both, AAB testing and the analysis of HLA markers.

PS 2

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MALARIA, GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AND TYPE 1 DIABETES IN SARDINIA

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Background and Aims: Malaria has been endemic in Sardinia from the prehistoric era up to its eradication in 1950. As a consequence of its positive selective effect, the prevalence of the Glucose-6-Phosphate Dehydrogenase deficient (G-6-PD⁻) phenotype in the Sardinian population is one of the highest in the Mediterranean area, and its geographical distribution in the island reflects that of past malaria occurrence. In the same population, childhood type 1 diabetes (IDDM) has the highest incidence in the world. As some reports have suggested a positive association between G-6-PD⁻ and diabetes (both type 1 and 2), we explored the association of IDDM with past malaria occurrence and with the G-6-PD⁻ phenotype. **Materials and Methods:** As it concerns past malaria morbidity vs IDDM, we calculated the correlation coefficient between the 1930's malaria morbidity rate and current IDDM incidence in 100 Sardinian communes. As it concerns G-6-PD⁻ vs IDDM, we adopted two parallel approaches: a) analysis of the geographical correlation between the G-6-PD⁻ phenotype prevalence rate and the 1990's IDDM incidence rate (age range 0-29 years) in 100 Sardinian communes; b) case-control study of the association between the G-6-PD⁻ phenotype and IDDM in 250 diabetic patients (140 males and 110 females, aged 15-29 years), as the cases, and 332 (163 males and 169 females) age- and sex-matched non-diabetic subjects from the same Sardinian area as the controls. **Results:** The G-6-PD⁻ prevalence rate ($\times 100$ residents) ranged 2.78-41.18 (median 18.7); IDDM incidence ranged 0-77.6, (median 27.0). The correlation coefficient between the two variables was $r = 0.12$ ($p = 0.24$). b) The Odds Ratio for IDDM associated with the G-6-PD⁻ phenotype was 1.25 (95% C.I.s 0.83-1.81) in hemizygous males, and 0.98 (95% C.I.s 0.50-1.70) in homozygous females. The correlation coefficient between IDDM and past malaria morbidity was $r = 0.039$ ($p = 0.70$, n.s.). **Conclusions:** Neither past malaria occurrence nor the ensuing high prevalence of the G-6-PD⁻ phenotype account for the high IDDM incidence among the Sardinian population.

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A NOVEL ASSOCIATION BETWEEN THE WOLCOTT-RALLISON GENE AND TYPE 1 DIABETES IN SOUTH INDIANS

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Background and Aims: The Wolcott-Rallison Syndrome (WRS) is a rare cause of neonatal diabetes inherited as an autosomal recessive. Mutations of EIF2AK3, encoding a translation initiation factor on chromosome 2, have recently been identified as the cause of WRS in two families. We have identified a South Indian family with a child with WRS but who had an age of onset of classical type 1 diabetes at 8 years, rather than in the neonatal period. Interestingly, the two siblings of the proband had type 1 diabetes, but no evidence of WRS, suggesting that they might be heterozygote carriers of the WRS gene. The South Indian family is only the eighth family to be described with WRS worldwide. We therefore hypothesised that the EIF2AK3 gene might determine susceptibility to T1DM in South Indians.

Materials and Methods: Two hundred and thirty six South Indian families consisting of an offspring with classical type 1 diabetes and both parents were typed for three single nucleotide polymorphisms (SNP) located within the EIF2AK3 gene using a PCR RFLP assay. Association and linkage between EIF2AK3 and type 1 diabetes was analysed by ETDI.

Results: Excess transmission of one of the SNPs was found (transmitted 97 times; not transmitted 65 times; $p = 0.012$); the other 2 SNPs were non significant ($p = 0.65$ and 0.73).

Conclusion: These preliminary results indicate that EIF2AK3 should be considered as a candidate gene for type 1 diabetes, at least in South Indians.

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MISSENSE MUTATIONS IN THE SUPEROXIDE DISMUTASE 2 (SOD2) GENE ASSOCIATED WITH TYPE 1 DIABETES

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Background and Aims: Type 1 diabetes (T1DM) is thought to be the polygenic disease. SOD2 gene, which is important to remove superoxides in mitochondria, locates in one of T1DM susceptibility regions, IDDM 5, and is therefore possible candidate gene of T1DM. **Materials and Methods:** Five exons of SOD2 gene were screened in 93 T1DM, 136 Type II (T2DM) and 117 non-DM unrelated subjects for mutations by PCR/SSCP and PCR/direct sequencing. **Results:** Two missense mutations, S27R and N153S, were identified, both in the heterozygous state, in only T1DM subjects. Serine at codon 27 is conserved among human, rat and mouse, and asparagine at codon 153 is conserved among human, rat, mouse and drosophila. The patients with S27R and N153S developed overt diabetes at the age of 15 and 7, respectively. ICA and ICSA were also positive. The subjects with S27R and N153S had T1DM susceptibility HLA DRB1-DQB1 haplotypes in Japanese on both alleles, 0405-0401/0901-0303 and 0405-0401/0802-0302, respectively. In the family of the patient with N153S, his father had the same mutation and T1DM susceptibility HLA haplotype on only one allele, 0405-0401/0803-0601. The father was not diabetic, however, his acute insulin secretion at early 30 minutes during 75g OGTT was impaired (Δ IRI30/ Δ BS30=0.39; normal >0.5). His mother and brother did not have the mutation. His mother had T1DM susceptibility HLA haplotypes on both alleles, 0802-0302/0901-0303, and his brother had the HLA haplotype on one allele, 0802-0302/0803-0601. They were not diabetic and their acute insulin secretion were normal (Δ IRI30/ Δ BS30=0.57 and 0.70, respectively). We are going to study the family of the patient with S27R. **Conclusions:** These results suggest that SOD2 may have an important role to protect pancreatic beta cells from oxygen free radical damage through the cascade of autoimmune events triggered by HLA in T1DM.

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ANALYSIS OF N ACETYL TRANSFERASE GENE POLYMORPHISMS IN ROMANIAN TYPE 1 DIABETIC FAMILIES.

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Background and Aims: Type 1 diabetes (T1DM) is a chronic disease with autoimmune pathology, determined both by genetic and environmental factors. Several reports suggest a role of nitrates intake (after conversion in N-nitroso compounds by N Acetyl Transferases - NAT) in diabetes pathogeny. Thus, NAT genes are functional candidates for T1DM. Polymorphic sites were described for NAT, homozygotes wild type being rapid, while those with at least one mutation slow, acetylators. Our aim was to assess the possible role of NAT2 gene (chromosome 8p21.3-p23.1) in T1DM genetic susceptibility for the Romanian population. **Materials and Methods:** We typed 341 T/C, 590 G/A, 803 A/G and 857 G/A polymorphisms of the NAT2 gene by Sequence Specific Primer PCR (SSP-PCR) in 204 Romanian T1DM families. The study group comprised 756 individuals: 212 diabetic probands (106M/106F, age at onset between 0-43 yr., median age at onset 12.1±6.7 yr.) and 544 unaffected parents and siblings. Allele transmission to affected and to unaffected siblings was determined by Transmission Disequilibrium Test (TDT). **Results:** We found a significantly increased transmission of 590 G allele to diabetics (92 T/61 NT, 60.1% transmission, $p=0.0075$) compared to their unaffected siblings (51 T/51 NT, 50% transmission). We found an increased transmission of 341 C (104 T/85 NT, 55% transmission, $p=0.095$) and 803 G (101 T/83 NT, 54.9% transmission, $p=0.1$) alleles to diabetics, compared with their unaffected siblings (56% transmission, $p=0.11$ for 341 C; 52.9% transmission, $p=0.29$ for 803 G). The transmission of 857 A/G alleles was not different than the expected 50%. **Conclusion:** We found a significantly increased transmission of 590G allele of the NAT2 gene to diabetics. These results require replication in a larger data set before any further conclusions can be drawn. If confirmed, this will strongly sustain the role of nitrates and N-nitroso compounds in T1DM pathogeny.

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IL12B and type 1 diabetes in Italian population: a case-control study.

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Background and Aims: It has been recently shown that a single nucleotide polymorphism at the 3'-UTR of the interleukin 12B (IL12B) gene is strongly ($p=10^{-4}$) transmitted to type 1 diabetes (T1D) children in a mixture of British and Australian families (Morahan). We tested whether the same IL12B variation is associated with type 1 diabetes in the Italian population

Materials and Methods: We typed the IL12B 3'-UTR A/C variation by PCR-RFLP in 274 Italian type 1 diabetes sporadic patients and 370 random controls. PCR primers were described in Huang et al. The presence of allele C creates a Taq I restriction site. Frequencies were compared by χ^2 heterogeneity tests.

Results: Gene frequencies were: A allele 69.2% (379 out of 548 chromosomes) and C allele 30.8% (169/548) in T1D subjects; A allele 72.3% (535/740) and C allele 27.7% (205/740) in Italian controls. Frequencies of genotypes AA, AC and CC were respectively: 47.1% (N=129), 44.1% (N=121) and 8.7% (N=24) in diabetics and 53% (N=196), 38.6% (N=143), 8.4% (N=31) in controls. Genotypes were in Hardy-Weinberg equilibrium in both cases and controls. Allele, phenotype and genotype frequencies did not significantly differ between T1D and healthy subjects.

Conclusions: A slight, not significant, increase (+3.1%) of the C allele was observed in diabetics compared to controls. This result goes in the opposite direction to what is described by Morahan and Huang (the more frequent, TaqI uncut A allele is transmitted to diabetics). If we hypothesize that the presence of the C allele (47% in Italian controls) confers a relative risk of 1.5, our dataset has 81% power to detect such effect at a statistically significant level (0.05). Thus we can exclude that the IL12B 3'UTR A/C polymorphism has a low-moderate (RR 1.5) effect in T1D in the Italian population.

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Genetic and Immunological analysis of 9 cases of Japanese nonautoimmune type 1 diabetes mellitus.

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Background and Aims: Imagawa et al. defined abrupt-onset type 1 diabetes characterized by the absence of diabetes-related antibodies and high serum pancreatic enzymes as nonautoimmune fulminant type 1 diabetes (N.Engl.J.Med.342:301,2000). We screened type 1 diabetic subjects in Ehime area in Japan, and found nine patients who had clinical characteristics of nonautoimmune fulminant type 1 diabetes. The aim of this study was to examine the genetic, immunological, and further clinical features of these patients. **Materials and Methods:** The HLA-DRB1 and -DQB1 were typed by PCR-RFLP. ICA was determined by indirect immunofluorescence method. GAD, IA-2 and other autoantibodies were assayed with the commercial kits. **Results:** These patients had no antibodies against either ICA, GAD, IA-2, thyroid gland, parietal cell, adrenal cortex or pituitary gland. Eight of these 9 patients were female, average age was 43.3±16.8 years. Mean duration of hyperglycemic symptoms was 2.8±1.3 days. At the initial examination, mean blood sugar was high (773±229 mg/dl), but mean HbA1c was close to the normal range (6.3±0.8 %). All the patient had ketoacidosis and lower urinary C peptide (2.8±1.3µg/day) and elevated serum amylase or elastase level. In three of these patients, the onset of diabetes was associated with pregnancy or delivery. Four patients had the HLA DRB1*0405-DQB1*0401, haplotype susceptible to type 1 diabetes in Japanese. One patient had both susceptible and protective haplotypes, and the two patients had protective haplotypes. The other one's haplotype was undetermined. **Conclusion:** These results suggest that nonautoimmune fulminant type 1 diabetes mellitus may be associated with pregnancy or the HLA DRB1*0405-DQB1*0401 haplotype.

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ANTI-BSA ANTIBODIES, BREAST FEEDING DURATION AND HAPLOTYPES IN TYPE 1 DIABETES IN CHILE.

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Type 1 diabetes is an autoimmune disease developed in genetically susceptible individuals and triggering by several environmental factors. Among them, breast feeding and the early exposure to cow's milk proteins seems to be important in the mechanism of β destruction. **Objective:** To evaluate the anti-BSA antibodies distribution in children with recent diagnosis of type 1 diabetes and control children from Chile. **Patients and Methods:** We studied the antibodies profile, risk HLA alleles and exclusive breast feeding history in 85 children with type 1 diabetes (age 8.1 ± 4.4 yr) and 58 control children (age 8.8 ± 2.9 yr). Anti-BSA by means of ELISA standardized in our laboratory, alleles typing by means of PCR and breast feeding by face to face interview. Wilcoxon and Chi-square test as statistical analysis.

Results: The exclusive breast feeding was lower in patients compared with the control children 4.3 ± 3.8 vs 5.5 ± 3.2 months ($p < 0.05$). Children with recent diagnosis of type 1 diabetes shown a higher levels of anti-BSA antibodies compared with non diabetic children 61.2 ± 7.7 ng/ml vs 4.00 ± 7.4 ng/ml ($p < 0.00001$). Table N°1 summarized the distribution of the BSA antibodies and the risk HLA (+): DQB1*0201.*0302 or protective HLA (-): DQB1*0301.*0602 in cases and controls.

	Cases (n=85)		Controls (n=58)		p-value
	N	freq	N	freq	
HLA + and BSA +	78	0.92	1	0.02	<0.00001
HLA + and BSA -	0	0.00	23	0.39	0.001
HLA - and BSA +	7	0.08	1	0.02	NS
HLA - and BSA -	0	0.00	33	0.57	<0.0001

Conclusion: These data suggest the presence of a possible interaction effect between HLA DQ risk alleles, the presence of low breast feeding period and high levels of anti-BSA antibodies.

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Perinatal factors and incidence of childhood-onset type 1 diabetes in a large population based cohort study

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Background and Aims: The causes of childhood diabetes are largely unknown and several studies have found perinatal factors associated to the risk of diabetes in children. The aim of this study was to estimate the associations of selected perinatal factors with the incidence of childhood onset type 1 diabetes in a large cohort study.

Materials and Methods: A cohort study by record linkage of the Medical Birth Registry and The National Childhood Diabetes Registry of Norway was performed. All live births in Norway between 1989 and 1998 (1 382 602 individuals) were followed for a maximum of 15 years, and they contributed a total of 8 166 731 person-years under observation in the period 1989-1998. Altogether 1824 cases of type 1 diabetes diagnosed 1989-1998 were identified within the cohort. The following exposure variables were included: maternal thyroid disease, pre-eclampsia, artificial induction of labour, bleeding during pregnancy, Rhesus-immunisation, placenta praevia, abruptio placenta, Caesarean section, twin or higher order multiple pregnancy and congenital malformations. Rate ratios were estimated from Poisson-regression analyses.

Results: There was a tendency that maternal thyroid disease [RR 1.62 (0.97, 2.69)] and Rhesus-immunisation [RR 1.95 (0.87, 4.34)] were associated with increased risk of type 1 diabetes, but the association was not statistically significant. Caesarean section, pre-eclampsia and the other perinatal factors included in the present study were not associated with risk of type 1 diabetes, neither was multiple pregnancy. Congenital malformations were not associated with risk of type 1 diabetes.

Conclusions: The majority of routinely recorded complications during pregnancy and delivery seems to be only weakly associated with incidence of type 1 diabetes, or not at all.

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HLA CLASS II GENES, INSULIN GENE POLYMORPHISM AND AUTOANTIBODIES IN SLOVAK CHILDHOOD DIABETES (DM1)

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Aims: This study aimed to evaluate HLA Class II alleles, insulin gene polymorphism and DM1 associated autoantibodies in Slovak children. **Material & Methods:** There were 152 DM1 patients (0-18 years) and 183 healthy controls of comparable age examined. HLA alleles were typed by PCR-SSP. Two insulin single nucleotide polymorphisms of the insulin gene (-23 Hph I; +1127 Pst I) were typed by PCR-RFLP. Autoantibodies to insulin (IAA), pancreatic tyrosin phosphatase (IA2-Ab) and glutamic acid decarboxylase (GAD₆₅) were detected by RIA. Odds ratios (OR) and positive predictive values (PPV%) were calculated. **Results:** Alleles DQB1*0302 (OR=5.16; PPV=0.03%), DRB1*0201 (1.55; 0.01%), DRB1*0404-14 (3.19; 0.021%), DRB1*0301 (2.57; 0.017%) and DQA1*0301 (1.61; 0.010%) as well as insulin gene polymorphism in homozygous carriers of -23 Hph I (OR>3) and those of +1127 Pst I (OR=2) were significantly ($p < 0.05$) associated with DM1 risk. Altogether the genetic risk pattern was present in 72% (65-79%; interval of 95% confidence) of 152 diabetics, while a relatively protective one in 23% (17-31% CI). Autoantibodies IAA were positive in 39%, IA2-Ab in 72% and GAD₆₅ in 73% of DM1 patients. **Conclusion:** This is the first comprehensive genetic and immunologic characteristics of Slovak children with diabetes mellitus type 1.

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ISOTYPES OF TYROSINE PHOSPHATASE-LIKE PROTEIN IA-2 AUTOANTIBODIES IN ADULTS AND CHILDREN WITH TYPE 1 DIABETES SHOW SIMILAR PROFILES.

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Background and Aims: Islet autoantibodies are known markers of type 1 diabetes with an immune-mediated basis and their isotype or subclass profile may also provide clues to changes of immune response during disease or after intervention. For ICAs and GADab, the IgG1 subclass consistently dominates in recent-onset disease. The aims of our study are to determine the isotype patterns for IA-2ab in Asian Chinese adults and children with type 1 diabetes.

Materials and Methods: From an initial screening of over 400 diabetes patients, 40 subjects (mean age 22.2 ± 15.8 yr) with IA-2ab were enrolled for this study. IA-2ab was detected by radioimmunoassay of ³⁵S-labelled recombinant human IA-2_{IC(605-979)}. Of them, 31 (median age 15 yr, range 2-57 yr; 16 children) had clinical type 1 diabetes (ie. requiring insulin at onset or within 1 year) with the majority having diagnosed recently (<1 year). The other 9 patients had clinical type 2 diabetes phenotype. **Results:** IA-2ab IgG subclasses determined with monospecific secondary antibodies, showed that both type 1 diabetes adults and children had similarly non-restricted isotype patterns with a strong presence of IgG1-IA-2ab. The rank order is IgG1>3>2>4; 15 subjects had detectable IgG4-IA-2ab. Clonality of immune response determined with lambda/kappa chain-specific antibodies also showed a non-restricted pattern. Patients with type 2 diabetes (38.2 ± 15.2 yr) had broad patterns of isotypes - IgG1/3 were detected more frequently (n=8) than IgG2/4 (n=5). Of three patients on insulin treatment, one was also positive for GADab. The remaining 6 patients were on oral hypoglycaemics. IA-2ab in type 2 diabetes were of low titre compared to type 1 diabetes. **Conclusions:** Isotype responses to IA-2 also had a strong IgG1 presence, similar to ICAs and GADab. With IgG3 subclass representation, a predominant Th1 milieu in the systemic environment is likely. There is no suggestion of differences in immune response to IA-2 between adults and children with type 1 diabetes.

PS 3

Type 2 Diabetes – Genetics: Genome Scans and Heritability

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Genome-wide scan for type 2 diabetes genes in Japanese

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Background and Aims: Genetic factors contribute to the development of type 2 diabetes mellitus. We have carried out a genome-wide screen for type 2 diabetes genes in the Japanese population as a first step in the identification of the genes contributing to the development of type 2 diabetes in this population.

Materials and Methods: The study population consisted of 256 affected sib pairs (379 diabetic subjects from 164 families) recruited from among the patients attending the Diabetes Clinic of Tokyo Women's Medical University and associated hospitals. The clinical features of the study population are: age-at-diagnosis, 45.3 ± 10.9 years (mean ± SD); HbA1c, 7.7 ± 1.7%; and BMI, 23.0 ± 3.0 kg/m². DNA was prepared from blood and genotyped using a panel of 414 markers from chromosomes 1-22. We tested each marker for linkage with type 2 diabetes using both two-point (programs SIBPAIR and SPLINK) and multipoint (GENEHUNTER-PLUS) methods of analysis. Results: We found five regions that showed nominal multipoint evidence of linkage with type 2 diabetes (i.e. MLS > 0.74, P=0.05). These regions were on chromosomes 2 (152-167 cM from pter), 4 (92 cM), 6 (33 cM), 9 (3-21 cM) and 21 (39-52 cM). The region on chromosome 9 (3-21 cM) showed suggestive evidence for linkage with MLS = 3.5 and pairs weighed equally.

Conclusions: Our genome-wide screen has revealed possible locations of genes that may contribute to the development of type 2 diabetes in the Japanese population. These results provide a focus for further genetic studies of type 2 diabetes in Japanese and the identification of genes that increase the risk of type 2 diabetes in this population.

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Annotating draft human genome sequence to aid susceptibility gene discovery in type 2 diabetes

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Background and Aims: Identification of type 2 diabetes susceptibility genes within the large chromosomal regions arising from genome-wide scans for linkage (often 10-20cM) remains a daunting task. The human genome sequence represents a valuable resource for positional cloning endeavours provided the information it contains can be systematically organised, annotated and "mined". We have developed a genome annotation tool (GANESH) and used it to aid gene discovery within a chromosomal region strongly-suspected of harbouring a diabetes-susceptibility locus. **Material and Methods:** GANESH combines various approaches (eg GENSCAN exon prediction; similarity searching against DNA and protein databases; cross-species comparisons) to identify putative regional transcripts within human sequence assembled from public sources. For each predicted transcript, attribute data (eg relationship to linkage and linkage disequilibrium data, qualitative and quantitative expression profiles, protein function prediction, metabolic role) are collected in automated manner and stored in a relational database amenable to complex queries. These assessments of the positional and biological candidacy of regional transcripts allow prioritisation of the strongest regional candidates for detailed study. **Results:** We have analysed the 1q21-24 region linked to diabetes in Pima, French, Mormon and UK families. Using GANESH, available public sequence for the region (from AP002532 to AL022310, ~20Mb) identifies 133 known genes including several strong candidates (eg RXRG, LMNA, KCNJ9). We also identified a further 145 putative transcripts with homology to known genes, and 393 "possible expressed" sequences predicted by GENSCAN, mouse genome comparison or EST similarity alone. GANESH also automatically identifies regional SNPs through matches to dbSNP: a total of 9590 SNPs were mapped within the region (~1 every 2kb). Collection of attribute data for these predicted genes is focusing initially on tissue expression patterns from known libraries of EST hits and functional annotation via InterPro domain content. **Conclusions:** Given the size of linked regions and the costs of SNP-typing, automated bioinformatics tools have an important role in allowing the strongest positional candidates to be identified. GANESH, and our methodologies for gene attribute collection, provide such a solution, which we expect to hasten identification of disease genes by providing a systematic, comprehensive framework for analysis of large chromosomal regions.

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DETECTION OF HUMAN OBESITY-ASSOCIATED POLYMORPHISMS BY AFLP GENOME SCAN.

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Background and Aims: Amplified fragment length polymorphism (AFLP) is a DNA fingerprinting technique widely used in plant and bacterial genetics, & also applied to mammalian disease and quantitative trait loci (QTL) genome scans. We used this technique to perform a genome scan in individuals from the Pacific island of Nauru to identify obesity-related polymorphisms.

Materials and Methods: Unrelated subjects were ascertained by Nauruan ethnicity, age 30-60 yrs. We performed AFLP on Mse I & Eco RI digested, & adapter-ligated, pooled genomic DNA stratified by sex & body mass index (BMI) into six groups: A. male 26.9(21.9-30.0), female 27.7(22.4-30.0); B. male 33.0(28.4-36.9), female 34.8(28.2-41.8); C. male 52.8(46.5-69.2), female 55.1(46.3-73.8); [ave.BMI(range)]. We used 19 pairs of selective primers resulting in 361 primer combinations each detecting 80-100 amplified fragments, approximating to a 1cM genome scan. Differentially amplified bands were cloned, sequenced, BLAST searched, flanking primers designed and then screened for polymorphisms. **Results:** 25 AFLP bands were differentially amplified between the phenotypic groups. So far we have identified & genotyped 4 single nucleotide polymorphisms (SNPs) from our selected bands: OBN11C1-4, OBN27C3, OBN41, & OBN25. We found no significant evidence for a difference in allele frequency or genotype distribution between our groups for the OBN11C1-4 SNP, or between female groups for the OBN41 SNP. However, we found significant evidence for differences in allele frequency between: all groups at OBN25 SNP (A. 0.82 vs B. 0.90 vs C. 0.95) P=0.038; all groups at OBN27C3 (A. 0.56 vs B. 0.71 vs C. 0.83), P=0.002; & male groups at OBN41 SNP (A. 0.85 vs B. 0.57 vs C. 0.56), P=0.002. (Fisher's Exact Test, df=2). We are currently fine-mapping these regions to identify obesity-associated genes. **Conclusions:** We have found evidence for the association of three different genomic regions with obesity in our Nauruan population, further characterisation of which will lead to better understanding of the metabolic abnormalities that are relevant to this pathogenic process. We also demonstrate the potential of AFLP as a useful technique in disease-gene discovery.

This research is funded by Autogen Ltd.

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The Benefits of the Thrifty Genotype

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Background and Aims: The thrifty genotype hypothesis suggested that the predisposition to Type 2 diabetes (Type 2 DM) was genetically determined and associated with increased survival at times of reduced energy intake. This postulate was based on the high prevalence of Type 2 DM in societies who had undergone periods of energy restriction in the past. Those with the thrifty genotype would be more likely to survive a period of famine leading to an increased frequency of the genotype. In recent times when food supplies have become assured and episodic famines have disappeared in the developed world such a trait would predispose to the development of obesity and Type 2 DM. No advantage was suggested for the development of actual diabetes which may have its deleterious effects beyond the reproductive years, or of gestational diabetes (GDM) which may be associated with foetal macrosomia. The mechanism of the thrifty genotype hypothesis may relate to a reduction in overall energy expenditure (demonstrated to be 0.5-1%) or during pregnancy to increase maternal triglyceride with an associated increase in birthweight which would in turn be associated with reduced perinatal mortality (PNM). We aimed to investigate in a computerised model of population dynamics, the relative benefits of these two putative advantages of the thrifty genotype.

Materials and Methods: The population distribution was based on early 20th century South Indian Asian data (birth rate 50 per 1000). Benefit through life of increased chance of survival through reduced energy expenditure was compared with reduction of PNM conferred through increased birthweight, in a population of whom 5% were termed thrifty and given either reduced total death rate or reduced PNM.

Results: Given an overall 0.1% benefit, the thrifty population increased to 5.57% of the total, 0.5% advantage increased to 8.02%; whereas a 0.1% disadvantage to the control population caused the thrifty population to increase to 6.61% of the total. A five-percent reduction in PNM caused the thrifty population to increase to 5.60% and a 10% reduction in PNM caused the thrifty population to increase to 6.17%. Given a 0.1% advantage through the whole thrifty population and a 10% reduction in PNM with an increase in maternal mortality to 0.7% the thrifty population increased from 5.00% to 6.75% of the total over 100 years.

Conclusions: The thrifty genotype may have a conferred advantage both through reduced energy expenditure for the whole population and increased chance of survival for the heavier infant born to a mother with predisposition to diabetes.

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Metabolic disorders in adult offspring whose both parents have obesity and hypertension.

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Background and Aims: Our earlier study concerning the familial aggregation of metabolic syndrome revealed the prevalence of hyperinsulinemia and hypo-HDLemia in non-obese, non-diabetic school aged children with positive family history. The aim of the study was to evaluate the prevalence of metabolic disorders related to insulin resistance in adult offspring whose both parents had obesity and hypertension and to compare it with the results of a group with negative family history. **Material and Methods:** The investigation was performed among subjects aged 40-55- the first group in countryside, the second in the town. The study group was drawn from a representative sample of population of Lublin region selected according to the epidemiological protocol. The examination was conducted in the town in groups: I-1 (n=20) obese with positive family history, I-2 (n=22) obese with negative history in parents, II-1 (n=17) non-obese with positive family history and II-2 (n=20) non-obese with negative family history. The same structure of investigated groups from countryside was named: III-1 (n=40), III-2 (n=22), IV-1 (n=41) and IV-2 (n=35) respectively. In all subjects we measured height, weight, blood pressure and we performed oral glucose tolerance test. We estimated fasting and 120' after load of 75g glucose serum insulin and blood lipids. We calculated body mass index (BMI) and insulin resistance index using Homeostasis Model Assessment (HOMA). **Results:** The mean serum fasting insulin in subgroups in the town was 10,6 sd= 5,7, 11,7 sd= 8,9, 6,6 sd= 6,2, 5,4 sd= 3,7 μ U/ml respectively and the mean postprandial insulin concentration was 34,7 sd= 19,5, 58,8 sd= 34,4, 43,7 sd= 66,8, 31,8 sd= 26,7 μ U/ml respectively. The mean value of IRI in these groups was 2,34 sd= 1,27, 2,71 sd= 2,02, 1,58 sd= 1,38, 1,26 sd= 0,83 respectively. The same direction of changes was observed in investigated subgroups in countryside but on the lower level. IRI in this subgroups was 2,05 sd=2,04, 1,97 sd= 1,48, 1,31 sd= 1,44, 0,7 sd= 0,39 respectively. The mean concentration of HDL cholesterol increased both within groups from I-1 to II-2. and III-1 to IV-2. **Conclusions:** It seems that there is a higher tendency for insulin resistance in the group of subjects with positive family history of polymetabolic syndrome in parents. It relates both to the obese and non-obese subjects. The lower insulin resistance observed in all the studied subgroups in the countryside probably results from a different lifestyle of this population.

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FATNESS AND FITNESS: THE IMPACT OF FITNESS ON THE GENETIC VARIATION IN TOTAL AND ABDOMINAL FAT

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Background and Aims: High measures of total and central abdominal fat and low level of physical fitness are predictive of the development of type 2 diabetes. The aims of this study are 1) to elucidate the relative importance of genetic and environmental factors on variation in total and abdominal fat, and 2) to assess the effect of adjusting for fitness on the total and the additive genetic variation of these two fat measures.

Materials and Methods: 152 female (79 MZ, 73 DZ) and 103 male (58 MZ, 45 DZ) twin pairs of 18-57 years underwent anthropometric measuring and a fitness test. Total fat in percent (fat%) was assessed by BMI, weight and age. Waist circumference was used as a measure of abdominal fat.

Results: Multivariate analysis methods were applied to these two measures of fat, adjusting for age and fitness. Waist circumference was also adjusted for total fat% to investigate abdominal fatness independently of overall fatness. Adjusting for age, the heritability of fat% was about 70% in both genders. Adjusting for age and overall fatness, the heritability of waist circumference was about 50% in both genders. Adjusting for fitness reduced the total variance of fat% by one third, and most of this reduction was in the additive genetic component. However, the fraction of the total variation due to the genetic variation (the heritability) changed very little. The total variation of waist, adjusted for overall fatness, was not reduced after adjusting for fitness in both genders.

Conclusions: A large proportion of the high heritability of total body fat(%) is due to genes implicated in fitness. There is little or no association between abdominal fatness and fitness.

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GLUCOSE TOLERANCE, INSULIN SECRETION AND INSULIN RESISTANCE IN PARENTS OF WOMEN WITH POLYCYSTIC OVARY SYNDROME

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Background and Aims: Insulin resistance with increased risk of type 2 diabetes is a common feature of polycystic ovary syndrome (PCOS). However, the antecedents of metabolic disorders in family members of patients with PCOS has been scarcely documented in the literature. Our objective was to evaluate glucose tolerance, insulin secretion and insulin resistance in parents of patients with PCOS compared to those of healthy women. **Materials and Methods:** A total of 120 parents of women with clinical and hormonal evidence of PCOS (PCOSp) and 74 parents of healthy normally cycling women (HWp) were studied. A 75-g oral glucose tolerance test (OGTT) was performed, and subjects were classified according to the ADA criteria. Glucose and insulin were measured at 0min before and 30, 60 and 120 min after the glucose load. C-peptide was also measured at 0 min. Total insulin secretion was assessed by the area under the curve of insulin (AUCI) during the OGTT, and insulin resistance, by HOMA model. For data analysis, all diabetic subjects were excluded. **Results:** The risk of developing diabetes was 2.4 (1.0-5.6) fold higher in PCOSp compared to HWp. Fasting insulin and fasting C-peptide concentrations were significantly higher in PCOSp compared to HWp. Total AUCI and HOMA (IR) were also significantly higher in the PCOSp group compared to the HWp group.

	HWp	PCOSp	p
Fasting insulin	11.86 \pm 7.1	15.44 \pm 11.5*	0.0280
Fasting C-peptide	1.30 \pm 0.83	1.95 \pm 0.92*	<0.001
AUC insulin	8714 \pm 5804.5	11179 \pm 8857*	0.0400
HOMA-IR	2.69 \pm 1.77	3.58 \pm 3.02*	0.0370

Conclusions: These data suggest that the parents of women with PCOS exhibit insulin resistance and type 2 diabetes more frequently than those of healthy women, thus constituting a risk group but an ideal population to detect and prevent the development of type 2 diabetes. Fondecyt grant 1000973.

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Insulin resistance is associated with sympathetic activation and blood pressure abnormalities in offspring of type 2 diabetic patients.

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Background and aims: Normotolerant offspring of type 2 diabetic patient are insulin resistant. It is therefore possible that some of the metabolic and/or haemodynamic abnormalities of type 2 diabetes may already be present in such subjects. Our aim was to investigate the relationship between insulin sensitivity, sympathetic activity and blood pressure profile in normotensive offspring of type 2 diabetic patients, with normal glucose tolerance.

Materials and Methods. 54 offspring and 10 control subjects were studied. They underwent an OGTT, impedance, 24h blood pressure and ECG monitoring, IVGTT followed by a euglycemic hyperinsulinemic clamp, with continuous blood pressure and ECG. Sympathovagal balance was evaluated as low- to high-frequency ratio (LF/HF), by spectral analysis on R-R intervals. Blood pressure profile was evaluated as the mean of systolic (SBP), diastolic (DBP) blood pressure during the day, the night, delta was: (day-night/day) \times 100.

Results. A significant correlation was observed between insulin-stimulated glucose disposal (M) and either night SBP (p<0.05) or DBP (p<0.04). According to the values of M, offspring were divided into 3 tertiles and the first tertile (resistant= R) was compared to the third tertile (sensitive= S). The two groups were similar for age, sex, body composition, BMI, W/H ratio. In R, a significant increase of both SBP and DBP during the night was present (night SBP: 116.5 \pm 2.5 vs S: 106.4 \pm 2.3, p<0.005; night DBP: 69.8 \pm 2.6 vs S: 61.8 \pm 2, p<0.021), while no difference was observed during the day in either SBP or DBP. As a consequence, the delta was significantly lower in R vs S both for SBP (delta SBP: 8.3 \pm 1.3 vs 12 \pm 1.1, p<0.041) and DBP (delta DBP: 13.1 \pm 1.9 vs 18.2 \pm 1.3, p<0.03). LF/HF ratio, similar in the two groups at baseline, was significantly higher in R at the end of IVGTT (4.1 \pm 0.7 vs I: 2.3 \pm 0.3, p<0.03) and did not change during the subsequent clamp study. On the contrary, in S, LF/HF ratio did not change at IVGTT but significantly increased throughout the following clamp (120 min: p<0.03 vs basal).

Conclusions. We confirm various degrees of insulin sensitivity in offspring of type 2 diabetic patients. In the sub-group of insulin resistant offspring, with normal glucose tolerance and normal blood pressure values, we now observe that insulin resistance is associated with abnormalities in blood pressure profile and with sympathetic activation. These data suggest that insulin resistant offspring already display some derangements of the autonomic nervous tone control, which may contribute to increase the incidence of hypertension and/or diabetes.

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INSULIN RESISTANCE: AN ATHEROTHROMBOTIC SYNDROME.

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Background and Aims. There is evidence to suggest that the phenotypic correlations observed between traits of the insulin resistance syndrome (IRS) may have a genetic basis. This study investigated evidence of pleiotropy between anthropometric, metabolic and haemostatic traits of the IRS. **Materials and methods.** Using the software package PAP (v4.0), we performed maximum likelihood analysis to investigate common genetic and environmental influences on traits of the IRS in 537 adults from 89 randomly ascertained healthy families of north European origin. **Results.** All traits showed significant heritability. There were significant genetic correlations (ρ_G), indicating pleiotropy between traits of the IRS (table). The genetic component explained 20-100% of the total phenotypic correlation (ρ_P). **Conclusions.** Pleiotropy between anthropometric, metabolic and haemostatic traits of the IRS is demonstrated in healthy white north European families indicating the atherothrombotic nature of this syndrome.

Trait 1	Trait 2	ρ_G	ρ_P	Genetic component \ddagger	Environmental component \S
Insulin	BMI	0.39*	0.45	0.13	0.32
	Triglyceride	0.31†	0.50	0.09	0.41
	Fibrinogen	0.42*	0.18	0.14	0.04
	Factor VII	0.30†	0.15	0.10	0.05
BMI	Triglyceride	0.29†	0.44	0.09	0.35
	sBP	0.63*	0.42	0.20	0.22
	PAI-1	0.49*	0.21	0.11	0.10
	Fibrinogen	0.52*	0.20	0.18	0.02
Triglyceride	HDL:C	-0.55*	-0.41	-0.19	-0.22
	PAI-1	0.59*	0.42	0.16	0.26
	Fibrinogen	0.36*	0.11	0.11	0

* $p < 0.01$

† $p < 0.05$

‡ $\rho_G \sqrt{h_1^2 h_2^2}$

§ $\rho_E \sqrt{(1-h_1^2)(1-h_2^2)}$

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MATERNAL TRANSMISSION OF TYPE 2 DIABETES: EVIDENCE FROM THE GOTO KAKIZAKI RAT MODEL

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Background and Aims: More than 20 studies have been published in the last decade investigating the pattern of disease inheritance in Type 2 diabetes. Most of these have reported an excess maternal transmission but, because it is a disease of late onset, parents of affected probands are usually not available for study and significant bias may result from historical recall of parental disease status. The few studies that have assessed parents directly have not confirmed significant maternal inheritance. The aim of the present study was to investigate any maternal effect in the inheritance of diabetes using a rodent model.

Materials and Methods: Female (diabetic) Goto Kakizaki (GK) rats (n=20) were mated with non-diabetic Wistar males, and male (diabetic) GK rats (n=20) were mated with non-diabetic Wistar females. Pregnancies were allowed to go to term and offspring studied after weaning. Tail-snip blood samples were taken after an overnight fast from all offspring for plasma glucose estimations at 6-weeks, 3-months and 6-months of age.

Results: A total of 149 offspring of diabetic (GK) mothers (maternal diabetes group) and 278 offspring of diabetic (GK) fathers (paternal diabetes group) survived to weaning. GK mothers tended to have smaller litters and a higher offspring mortality than Wistar mothers. At 6-weeks of age, the mean plasma glucose of the maternal diabetes group was 7.45 mM (+/- 0.62, standard error of mean) and of the paternal diabetes group was 7.50 mM (SEM 0.45) (p=ns). At 3-months of age, mean plasma glucose of the maternal diabetes group was 7.03 mM (SEM 0.6) and of the paternal diabetes group was 7.21 mM (SEM 0.49) (p=ns). At 6-months of age, mean plasma glucose of the maternal diabetes group was 5.60 mM (SEM 0.48) and of the paternal diabetes group was 6.07 (SEM 0.4) (p=ns).

Conclusions: Plasma glucose levels of offspring were not influenced by whether the mother or the father had diabetes. Further studies (e.g. intravenous glucose tolerance and insulin measurements) will investigate whether more subtle differences exist between the offspring of diabetic mothers and fathers.

PS 4

Type 2 Diabetes – Genetics: Candidate Genes in Insulin Secretion

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A986S polymorphism of the calcium-sensing receptor, insulin secretion and metabolic syndrome in women with prior gestational diabetes.

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Background and Aims: Calcium-sensing receptor, which is also located on the surface of the pancreatic B-cell could modulate insulin secretion in vitro. Disturbance of insulin secretion is one of the major causal factors in the development of gestational diabetes (GDM) that supposed to be an early manifestation of the metabolic syndrome. To study the potential role of the calcium-sensing receptor in GDM its A986S polymorphism was investigated.

Materials and Methods: The polymorphic region was amplified by allele specific PCR technique. The A (containing alanine) and S (containing serine) alleles were determined in 62 women with recent or prior (n=49) GDM. Type 1 (n=55), type 2 (n=25) diabetic patients and healthy persons (n=201) served as controls. Association between reclassification data (blood glucose, IRI, C-peptide and leptin values during a 75g oGTT, HbA1c, lipoprotein lipids, PAI 1, fibrinogen, BMI, waist-to-hip ratio, blood pressure, fat %, creatinine and uric acid) of prior GDM women (mean age: 35.8 ± 5.7 [±SD] yrs; BMI: 27.8 ± 7.1 kg/m²; time elapsed since delivery: 3.4 ± 0.4 yrs; 46% glucose intolerance [GI = diabetes mellitus or IGT]) and the genotypes of the A986S calcium-sensing gene were investigated using ANOVA and t-tests for statistical analysis.

Results: Genotype frequencies were 68% AA, 29% AS and 3% SS. No significant differences were found between any of the investigated groups. The genotypes were divided into presence (32%) and absence (68%) of S allele. The absence of S allele significantly correlated with the fasting (P=0.025) and 60 min glucose values (P=0.008) and inversely with the 30 min C-peptide levels (P=0.022) during oGTT. Fasting leptin (P=0.026), PAI 1 (P=0.011) and LDL-cholesterol (P=0.015) values were also associated inversely with the absence of the S allele.

Conclusions: According to our results the presence of the A alleles might protect against hyperinsulinaemia and prevent the development of the metabolic syndrome. Characteristic features of the latter (elevated LDL-cholesterol, PAI 1 and fasting leptin values) were found in the presence of the S alleles.

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INSULIN GENE VNTR ASSOCIATED WITH TYPE 2 DIABETES: INTERACTIONS WITH AGE, BODY FATNESS AND HEIGHT

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Background and Aims: Transcription of the insulin gene is influenced by a VNTR polymorphism, with two main alleles: class I and III. Associations of the class III allele with type 2 diabetes mellitus have been shown, but not consistently. Gene-environment interactions could explain these inconsistencies. **Materials and Methods:** We investigated whether variation at the INS VNTR locus was associated with the prevalence of diabetes mellitus, diagnosed by an oral glucose tolerance test, in 438 men aged 70-89 yrs (the Zutphen Elderly Study), taking into account age, body fatness and height. **Results:** Eleven percent of the men were homozygous for the class III allele. The prevalence of newly diagnosed diabetes was higher in this group (OR: 1.99, 95% CI 0.82-4.82) compared to the I/I and I/III subjects. The association was stronger in younger men (70-74 yrs, OR: 3.22, 95% CI 1.14-9.04) than in older men (75-89 yrs, OR 0.65, 95% CI: 0.08-5.29) (p multiplicative interaction 0.02). Higher than expected OR's (additive interaction) were also seen in shorter men (<median 172 cm) (OR 3.09, p=0.04) and in men with subscapular skinfold thickness above the median (OR 2.19, p=0.07). Analysis of the patterns of interaction showed that for age and height both the risk factor and the INS VNTR variant class III were required to raise the risk of diabetes. No clear interaction between the INS VNTR genotype and BMI was observed. **Conclusions:** The association between INS VNTR and diabetes is less clear in the oldest old. In contrast to overall body fatness, central body fat appears to elevate the risk of diabetes only in subjects with the class III allele. The interaction between INS VNTR and body height may be explained by a reported interaction with intrauterine development.

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FAMILIAL HYPERINSULINEMIA CAUSED BY ABNORMAL INSULIN (INSULIN WAKAYAMA)

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We recently identified a new family from Kishiwada, Japan with abnormal insulin. A 65-year-old non-obese Japanese man with diabetes mellitus had marked hyperinsulinemia with elevated IRI/CPR molar ratio. Achromatosis nigricans was not detected. Laboratory tests were normal except for marked fasting hyperinsulinemia (130 μ U/ml). Counterregulatory hormone was within normal limit. Anti-insulin and insulin antireceptor antibodies were absent. Family study revealed that his daughter also showed hyperinsulinemia. She was 31-year-old and had normal glucose tolerance. Proband's elder brother, who was 72-year-old, was not diabetic without hyperinsulinemia. DNA analysis confirmed that proband and his daughter demonstrated the identical point mutation leading to the expression of Leu A3 insulin previously described for Insulin Wakayama. His daughter secreted insulin about two times as much as the proband in postprandial conditions and after load of 75g glucose. Peak time of blood IRI was 120 minutes after load in 2 hour 75g oral glucose tolerance test. All abnormal insulins discovered in Japan were insulin Wakayama. This family was fourth family of insulin Wakayama in the world.

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PARENT OF ORIGIN EFFECT AT THE INSULIN GENE LOCUS: COMMON GENETIC MECHANISM MODULATING DISTURBED EARLY GROWTH AND RISK OF TYPE 2 DIABETES.

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Background and Aims: There is growing evidence that the complex association between early growth and metabolic phenotypes in later life is, in part, due to the pleiotropic effects of genes modulating insulin secretion and/or action, and thereby contributing to variation in both fetal and adult phenotypes. One such locus is the insulin gene. Family-based association studies have shown that INS-VNTR-associated susceptibility to type 2 diabetes is transmitted preferentially through paternal class III alleles, and is, by implication, mediated through imprinted mechanisms active in early life. This is supported by a recent study which demonstrated maternal imprinting and paternal expression of the insulin gene in the human yolk sac. Evidence for similar imprinting effects modulating the known association between INS-VNTR class and early growth would therefore provide further support for a common genetic mechanism.

Materials and Methods: We examined INS-VNTR genotypes of 191 European parent-offspring trios, each ascertained via unselected consecutive births. Measurements of infant weight, length and head circumference were available (expressed as SD scores, using sex specific UK Reference Data) at 0, 6 and 12 months of age. Parental and cord-blood samples, were genotyped for the -23HphI variant (which acts as a surrogate for VNTR class), with ambiguities in the parental origin of fetal alleles resolved through additional INS-VNTR class I typing. Our search for imprinting effects focussed on the 51 heterozygous infants, 21 of whom had inherited a paternal class I, and 30 a paternal class III.

Results: No differences were seen at birth, but, by 6 months, there was a clear divergence in growth patterns, with the paternal class III babies both lighter (weight SDS (SD): 0.03(1.02) vs 0.66(0.90), $p=0.029$) and shorter (length SDS (SD): 0.13(1.00) vs 0.90(0.81), $p=0.006$) than those with paternal class I. In this study, we found no evidence for a relationship between fetal VNTR genotype (I/II vs III/III, $p>0.32$) and parameters at birth, in contrast with previous ALSPAC data (though numbers were smaller).

Conclusions: These data provide the first evidence that VNTR-associated effects on early growth, like those on diabetes, demonstrate parent-of-origin effects, lending further support for a genetic contribution to the link between fetal and adult phenotypes.

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CALCIUM / CALMODULIN DEPENDENT PROTEIN KINASE II GENES: GENOMIC STRUCTURE AND SCREENING FOR VARIANTS IN SUBJECTS WITH TYPE 2 DIABETES

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Background and Aims: CaM Kinase II, a multifunctional Ca^{2+} / calmodulin - dependent protein kinase, is expressed in the pancreatic beta-cell and is activated by glucose and other secretagogues in a manner correlating with insulin secretion. It has been proposed that the activation of CaM Kinase II mediates some of the actions of Ca^{2+} on the exocytosis of insulin. Thus, the genes encoding for members of this multigene family are important candidate genes for beta-cell dysfunction in Type 2 diabetes (T2DM). We aimed to determine the genomic structure of both the gamma and delta isoforms (CAMK2G and CAMK2D) and screen the exons and exon-intron boundaries for variants in subjects with T2DM. **Materials and Methods:** Genomic structure of the CAMK2G gene was determined by identifying 6 contiguous clones from a human P1 artificial chromosome (HPAC) library using a CaM Kinase II γ cDNA probe. Positive clones were confirmed as γ by PCR amplification of the γ specific variable domain VIII. Fluorescence *in situ* hybridisation (FISH) localised these clones to chromosome 10q22. The published genomic structures of the rat and mouse CaM Kinase II genes allowed the putative exon-intron boundaries of human CAMK2G to be identified. These were confirmed by vectorette PCR amplification and resequenced from genomic DNA. CAMK2D genomic structure was determined by sequence homology to genomic contigs in the NCBI database. DNA from 76 randomly selected subjects with T2DM was screened by SSCP analysis for variants in both genes. **Results:** CAMK2G was composed of 23 exons (43-123bp) whilst, CAMK2D consisted of 18 exons (42-122bp). Screening of both genes has identified variants; in CAMK2G a silent variant (K⁴⁴K) in exon 2 (AAA \rightarrow AA \bar{G}) which encodes for part of the catalytic domain and an intronic variant in exon 10 (+58c \rightarrow -A). In CAMK2D an intronic variant was detected in exon 14 (-45g \rightarrow e). **Conclusions:** We have determined the genomic structures of CAMK2G and CAMK2D, localised the CAMK2G gene to chromosome 10q22 and identified variants which can be used to determine the role of these genes in susceptibility to T2DM.

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SEARCH FOR MUTATIONS OF THE Ca^{2+} /CALMODULIN-DEPENDENT PROTEIN KINASE II δ IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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The Ca^{2+} /calmodulin-dependent protein kinases II (CaMK II) are multifunctional intracellular enzymes which take part in the glucose-stimulated insulin-secretion. Mutations of CaMK II may lead to an impaired insulin-secretion and could play a key role in the multifactorial pathogenesis of the development of type 2 diabetes mellitus. Human β -cells show a high expression-level of CaM-kinases and CaMK II β and δ are the predominant isoforms when compared to CaMK II γ while the α -isoform was not expressed. The aim of this study was the detection and sequencing of the previously partial known cDNA-sequence of CaMK II δ . Furthermore mutation analysis of the CaMK II δ cDNA-sequence of type 2 diabetic patients was performed. **Methods:** RNA was extracted from lymphocytes of patients with type 2 diabetes mellitus and reverse transcribed into cDNA. Five primer pairs were designed for PCR according to a 727 bp sequence of the 5'-region of CaMK II δ which was cloned from a human insulinoma cDNA-library and from partial sequences which were obtained from GenBank. These 5 primer pairs cover the complete coding sequence. All PCR-products were directly sequenced at least twice and analysed for mutations. **Results:** PCR-amplificates of the five primer pairs were obtained from 36 patients with type 2 diabetes mellitus. All PCR-products had the expected size. Additional amplificates which represent splice variants were not found. Direct sequencing showed the expected sequence of CaMK II δ ; but no mutation. **Conclusion:** Although in mammals 12 subtypes of CaMK II δ are known, in this study only the δ_2 -subtype was detected in patients with type 2 diabetes mellitus, which showed no mutations of the coding sequence. If these findings play an pathophysiological role in the genesis of type 2 diabetes mellitus must be shown by further studies with more patients.

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TRANSCRIPTIONAL REGULATION OF THE -132 G/A MUTANT ISLET AMYLOID POLYPEPTIDE (IAPP) GENE PROMOTER

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Background and Aims: Overexpression of islet amyloid polypeptide (IAPP) has been implicated in islet amyloidogenesis. In previous studies in the Spanish population, we reported a -132 G/A mutation in the promoter region of the IAPP gene. The frequency of this mutation was higher in type 2 diabetic patients than in non-diabetic subjects (9.7% vs. 1.5%, $p < 0.005$, odds ratio: 6.85). We aimed to study the functional properties of the G/A mutant IAPP promoter, and to compare the effects of glucose metabolism, calcium, cAMP, and dexamethasone on the activity of the mutant and wild-type IAPP promoter.

Materials and Methods: Experiments were performed in MIN6 β -cells. Plasmids were constructed containing the -229 to +458 bp region of the human IAPP promoter. Promoter activities were analysed by luciferase assay after 20-24h of culture in the presence of 5.5 or 22.7 mmol/l glucose, 11.2 mmol/l mannoheptulose, 11.2 mmol/l 6-deoxy-glucose, 0.6 mmol/l diazoxide, 100 μ mol/l verapamil, 10 μ mol/l forskolin, and 10 μ mol/l dexamethasone.

Results: The mutant construct showed a 2-fold increase in the IAPP promoter activity compared to the wild-type construct ($p < 0.001$). The luciferase activity of cells transfected with the wild-type construct incubated in 22.7 mmol/l decreased by 25% and 30% after the addition of 6-deoxy-glucose and mannoheptulose, respectively. A similar pattern was observed with the mutant construct, but with higher levels of activity. Both constructs showed a severe reduction in the promoter activity (4-fold decrease) after reduction of intracellular calcium concentrations in the presence of verapamil or diazoxide ($p < 0.001$). By contrast, the IAPP promoter activity was enhanced (2-fold increase) in both constructs after incubation with forskolin or dexamethasone ($p < 0.05$ and $p < 0.01$, respectively).

Conclusions: We have detected a -132 G/A mutation in the IAPP gene that increases IAPP expression. The mutant construct reproduces the same pattern of IAPP activity as the wild-type construct, but with higher values of activity. Glucose regulation of IAPP expression in both constructs requires a calcium signalling pathway preserved. The detection of an enhanced expression of IAPP after stimulation with forskolin and dexamethasone suggests the presence of functional cAMP- and glucocorticoid-responsive elements in the IAPP promoter, and that these factors contribute to the transcriptional regulation of the IAPP gene.

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Prevalence of Islet amyloid polypeptide promoter mutation (-132 G to A) in type 2 diabetes.

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Background and Aims:

Islet amyloid polypeptide (IAPP) forms islet amyloid in type 2 diabetes (T2DM). Over-expression of IAPP in association with IAPP gene mutations could be causal factors for amyloidosis. A mutation in the IAPP promoter at position -132 (G to A) has been described in T2DM (9.2%); 2.3% in non-diabetic (ND) subjects in Spain. The aim of the study was to determine the prevalence of this mutation in a UK population and to examine its relationship to amyloid formation in a kindred with T2DM.

Materials and Methods:

Samples were collected from 278 T2DM subjects and 110 unrelated ND subjects derived from the Oxford region. To investigate the relationship between this mutation and islet amyloid observed at post-mortem, five brothers (3 T2DM and 2 ND) with a family history of T2DM were studied. Two of the T2DM brothers had extensive islet amyloid at post-mortem examination following their death. DNA was analysed for the IAPP promoter mutation (-132 G to A) by SSCP and direct sequencing.

Results:

The IAPP promoter mutation was detected in 14/278 (5%) diabetics; 4/110 (3.6%) ND controls (not significant); and in 1 diabetic and 2 ND brothers, but not in one patient with amyloid.

Conclusions:

The IAPP promoter mutation is not associated with T2DM. There is no association with islet amyloid deposition in the family studied.

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Genetic mutation of HNF 1 α in Korean type 2 diabetes with familial traits

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Background and Aims: Type 2 diabetes mellitus is a heterogeneous, multifactorial disease with a strong genetic background. The most frequent genetic background worldwide is MODY 3 (maturity onset diabetes of young) related gene, especially hepatocyte nuclear factor 1 alpha (HNF1 α). HNF1 α is a transcriptional factor related with hepatocyte development, differentiation and insulin secretory function of beta cells. We performed DNA extraction and sequencing to determine whether HNF1 α gene mutation may contribute abnormal glucose metabolism in Korean type 2 diabetes with familial traits.

Materials and Methods: We evaluated HNF 1 α gene mutation by direct sequencing method in 17 Korean type 2 diabetes with familial traits (male 11: female 6) and 3 unrelated NGT control subject without familial traits of DM. We evaluated glucose metabolism with OGTT, beta cell function and insulin sensitivity index (ISI) by HOMA models. The familial traits were defined by 2 or more diabetic patients in the 1st degree relatives or 3 or more diabetic patients in the 2nd degree relatives.

Results: Three new genetic mutation (V259I, H126A, S121T) and one polymorphism (119 valin GTC->GTG) in the HNF 1 α were found in Korean type 2 diabetes with familial traits. One family had a common mutation in all diabetic patients of the family subjects (exon 4 V259I, 2 cases). Also other mutations (H126A, S121T) were found in Korean type 2 diabetes with familial traits. The mutation group (n=5) revealed reduced beta cell function compared to the non-mutation group (n=12) ($p=0.052$).

Conclusions: We found new HNF1 α gene mutations in Korean type 2 diabetes population with familial traits. Those type 2 diabetic patients who have HNF1 α mutation revealed reduced beta cell function. We concluded that HNF 1 α gene mutation may contribute to glucose metabolism in Korean type 2 diabetes with familial traits.

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Mutations in HNF1-alpha as Risk Factor for Gestational Diabetes

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Background and Aims: Gestational Diabetes mellitus (GDM- diabetes mellitus type-4), is a heterogeneous disorder defined as glucose intolerance with first recognition during pregnancy. Mutation in the hepatocyte nuclear factor-1alpha (HNF-1alpha) gene cause MODY3, which is characterized by an impairment of insulin secretion. Amino acid polymorphisms in HNF-1alpha, for example the AV98 variant, are associated with reduced glucose-induced C-peptide and insulin responses. We have addressed the question of whether variants in the HNF-1alpha gene might be associated with gestational diabetes mellitus.

Materials and Methods: The coding region of the HNF1-alpha gene was analyzed in a case-control-study in 58 women with GDM and 63 controls with common PCR- and sequencing methods GDM was diagnosed with a 50g glucose screening test followed by a 75g OGTT test between 24 to 28 week of gestation. Obvious MODY-patients were excluded from patients group.

Results: Clinical characteristics of GDM patients and controls were: mean age $34.1 \pm 7.5/32.5 \pm 8.8$, (Mean \pm SD) GAD AB 13%/0%, INS AB 3%/2%, ICA 0%/0%, maternal diabetes 80%/36%). We identified nine heterozygous missense variants (Glu48Lys, AV98, Pro447Leu) in the HNF1-alpha gene and twice the insTGGGGGT variant in the 5'UTR. The patient with the Glu48Lys presented with low titer of GAD- and Insulin auto antibodies. This patients would be classified as type-1 diabetic. Seven patients (12%) carried the AV98 missense variant and had a maternal and grand maternal history of GDM and/or diabetes. The GDM patient with the Pro447Leu showed diabetes in four consecutive generations. None of the variants were seen in controls ($p=0.0048$).

Conclusions: Mutations in HNF1-alpha are a risk factor for gestational diabetes. About 17% of German gestational diabetic patients carry mutations in the HNF1-alpha gene. The Glu48Lys was previously seen in type-1 diabetes and might be involved in autoimmune diabetes. The previously described 30% reduction in glucose induced C-peptide and insulin secretion in patients carrying the AV98 variant can be pathogenetic for GDM.

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A BETA2/NEUROD1 POLYMORPHISM AS A DETERMINANT OF SUSCEPTIBILITY TO TYPE 2 DIABETES IN A SOUTH INDIAN POPULATION.

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Background and Aims: Beta2/NeuroD1 is a member of the basic helix-loop-helix (HLH) family of transcription factors, which is involved in pancreatic embryogenesis and regulating transcription of the insulin gene. Polymorphisms within the Beta2/NeuroD1 gene associate with increased susceptibility to Type 1 Diabetes and have been found in some families with autosomal dominant diabetes. This study investigated the role of a previously reported G to A transversion in codon 45 that results in an amino acid substitution (Ala45Thr).

Materials and Methods: 462 subjects from an urban survey were recruited as part of a study into the prevalence of Type 2 Diabetes (T2DM) in a South Indian population. Ala45Thr results in a loss of a MwoI restriction site. We screened for these substitutions by PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism), and then the DNA fragments were separated on a 3.2% agarose gel and visualised by ethidium bromide staining.

Results: An association study in the urban survey found a significant difference in the subjects positive for the variant allele between unrelated subjects with T2DM (n=84, 14.3% positive), IGT (n=59, 33.9% positive) and controls (n=319, 23.9% positive) for the Ala45Thr polymorphism (p=0.023). Quantitative trait analysis for waist hip ratio, body mass index, lipid profile, fasting blood glucose and 120 minute oral glucose tolerance profile for each of these groups revealed no significant association with Ala45Thr.

Conclusions: These preliminary findings suggest variation in the Beta2/NeuroD1 gene may contribute to glucose intolerance in a South Indian population.

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HOMOZYGOUS MUTATIONS IN NEUROD AND PAX4 IMPAIR INSULIN SECRETION IN PATIENTS WITH DIADETES

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Background and Aims: Heterozygous polymorphisms: the Ala45Thr of NeuroD and the Arg127Trp of Pax4 genes does not, reportedly, seem to be associated with type 2 diabetes. We discovered the homozygous mutations of these transcriptional factors in patients with, late-onset diabetes. To clarify the role of these mutations in the pathogenesis of this diabetes, we examined the insulin secretion and sensitivity in these patients. **Materials and Methods:** Association studies were carried out in 283 patients with diabetes excluding type 1A diabetes, age 61.9±11.9 years and age at clinical onset of diabetes 51.4±12.8 years and 134 unrelated control subjects, age 70.1±7.8 years. Genotyping for the Ala45Thr of NeuroD and the Arg127Trp of Pax4 genes was carried out by PCR-RFLP followed by the direct sequence method. Acute insulin secretion was evaluated by C-peptide secretion rate mathematically estimated by two-compartment model after intravenous glucose load (CS). Insulin sensitivity was evaluated by insulin-modified minimal model analysis (Si). **Results:** Genotype frequencies in patients and in control subjects were: the Ala45Thr of NeuroD (wt 0.809 hetero 0.177 homo 0.014 vs wt 0.896 hetero 0.104 homo 0.000) and the Arg127Trp of Pax4 (wt 0.915 hetero 0.074 homo 0.011 vs wt 0.955 hetero 0.045 homo 0.000). Homozygous mutations of both genes were observed only in the patients. In the patient A, B and C with homozygous mutation of NeuroD, CS (normal range: 6.8-18.5ng/ml/min) was 0.508, 1.481 and 1.223, respectively and Si (normal range: 2.6-7.6 × 10⁻⁴/min/(mU/L)) was 0.727, 3.31 and 3.79. In the patient D and E with homozygous mutation of Pax4, CS was 0.418 and 0.208 and Si was 1.11 and 2.88. These results indicated the markedly impaired insulin secretion in the patients with the homozygotes. **Conclusions:** Since both mutations were not located in the DNA binding and transactivation domains, the effect of the variants on the function may be weak and thus, the heterozygotes do not influence the β-cell function. However, the homozygotes impair the insulin secretion, inducing diabetes.

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PEROXISOME PROLIFERATORS ACTIVATED RECEPTORS IN TYPE II DIABETIC SUBJECTS

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Aim: PPARs have been shown to be involved in the regulation of intermediary metabolism (e.g., control of metabolic pathways important for lipid homeostasis, expression of proteins engaged in lipid transport, and adipose tissue differentiation). Isoforms α and γ seem to be implicated in the development of inflammation, atherosclerosis, metabolic syndrome and type II diabetes. We assessed the frequencies of PPARα isoform Leu162Ile (known as α*3) and on the γ isoforms: Pro34Ala (called *2) and Pro344Gln (*3), in diabetic and healthy subjects. **Methods:** To study PPAR isoforms and variants involved in type II diabetes, 104 patients with BMI≥30 and diagnosis of type-II diabetes <50 years and 261 control DNA samples were selected and enclosed in this study. Poly Chain Reaction (PCR), restriction enzyme digestions and sequencing of mutated alleles were performed for PPARα (variant *3) and PPARγ (*2 and *3) isoforms. **Results:** Entire genomic data are available for 235 controls and 98 patients and shown in the Table:

PPARs	Controls				Patients			
	%	n	Heteroz	Homoz	%	n	Heteroz	Homoz
alpha*3	4.3	258	18	2	2.9	104	6	0
gamma*2	8.6	251	41	1	6.5	100	13	0
*3	4.0	248	20	0	1.5	101	3	0

Conclusions: Cross-talk between α and γ PPARs subtypes could be important for type II diabetes etiopathogenesis. Finally, these results should be useful to determine the relationship between genotypes and phenotypes, using different types of mutations and clinical data.

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Association Of Pro12Ala Variant In Peroxisome Proliferator - Activated Receptor With Type 2 Diabetes Mellitus

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Background and Aims: To investigate the association of Pro12Ala variant in peroxisome proliferator-activated receptor-γ gene with type 2 diabetes mellitus and its clinical characteristics.

Materials and Methods: The genotypes of Pro12Ala variant in peroxisome proliferator-activated receptor-γ gene were determined by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) assay in 401 unrelated subjects of "Han" population in the south of China (including 180 subjects with normal glucose tolerance and 221 type 2 diabetic patients). The clinical data were also analyzed.

Results: The allele frequencies in case and control groups were 96.15%, 96.11% for P and 3.85%, 3.89% for A; the genotype frequencies were 92.77%, 92.22% for PP, 6.78%, 7.78% for PA, 0.45%, 0% for AA. The Pro12Ala variant of peroxisome proliferator-activated receptor-γ was not associated with type 2 diabetes. The Pro12Ala polymorphism of peroxisome proliferator-activated receptor-γ2 in diabetes patients was associated with increased waist circumference and waist to hip ratio. The Pro12Ala polymorphism in Chinese population was similar to that in Japanese population, and was different from that in European and American population.

Conclusion: The Pro12Ala variant of peroxisome proliferator-activated receptor-γ2 could be associated with abdominal obesity in type 2 diabetes. The significant difference of Pro12Ala of peroxisome proliferator-activated receptor-γ2 among various races was observed.

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THE Pro12Ala PPAR gamma2 GENE POLYMORPHISM IN CZECH TYPE 2 DIABETICS AND OBESE PATIENTS

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The peroxisome proliferator-activated receptors (PPARs) are the transcription factors. PPAR γ 2 plays a key role in regulation of adipocyte differentiation and energy homeostasis. Numerous recent studies provide the evidence that the Pro12Ala polymorphism is linked to obesity and type 2 diabetes mellitus but the results are controversial and depend on the ethnicity. The aim of this study was to determine allele frequencies and to study the influence of the polymorphism on biochemical and anthropometric parameters in healthy Czech adult population (n=69; age=32.6 \pm 10.2 years; BMI=23.9 \pm 3.7 kg/m²), in a group of type 2 diabetics (n=183; age=59.0 \pm 6.2 years; BMI=30.1 \pm 4.8 kg/m²) and in a group of obese women (n=86; age=44.1 \pm 11.4 years; BMI=37.5 \pm 5.8 kg/m²). **Methods:** The Pro12Ala substitution was detected by PCR-RFLP method (HgaI). For statistical analyses, the NCSS 2000 program was used. **Results:** χ^2 test did not reveal significant differences in Pro12Ala frequency between the group of diabetic patients and controls (hetero-, homozygotes: 26.23%, 1.09% vs. 15.94%, 1.45%, $\chi^2=2.66$; p=0.10). However, the occurrence of Pro12Ala tended to be higher in a group of obese women (29.07%, 1.16%) in comparison to the control group ($\chi^2=3.41$; p=0.06). The Mann-Whitney test revealed significantly lower fasting insulin and C-peptide levels in the Pro12Ala carriers in a group of diabetic women (12.17 \pm 7.76 vs. 17.66 \pm 11.49 mIU/l; p=0.006 and 0.89 \pm 1.01 vs. 0.91 \pm 0.43 nmol/l; p=0.02, resp.). Tendency to lower fasting insulin levels was also evident in diabetic men (14.79 \pm 11.82 vs. 16.46 \pm 10.72 mIU/l, n.s.). Taken all probands together, there were no differences in body constitution (BMI, WHR), body composition (%fat mass, %muscles, %bone mass), lipid levels or in other tested parameters between the Pro12Ala carriers and non-carriers. **Conclusion:** The frequency of the Pro12Ala PPAR γ 2 gene polymorphism in Czech probands is similar to other Central European populations. Frequency of the Pro12Ala substitution tends to be slightly higher in obese women compared to the controls. The fasting insulin and C-peptide levels in the Pro12Ala carriers were significantly higher in the group of diabetic women. This finding provides evidence that polymorphism may influence glucose metabolism.

Supported by grants IGA MII NB/5395-5, COST B17, GA UK 20303091/94.

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Analysis of the relationship between PPAR- γ 2 gene variants and severe insulin resistance in obese patients with impaired glucose tolerance

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Four mutations and one silent polymorphism in the peroxisome proliferator-activated receptor- γ 2 (PPAR- γ 2) gene have been identified. It was recently shown that loss-of-function PPAR- γ mutations are associated with elements of the insulin resistance syndrome. However, there is a Pro115Gln mutation of the PPAR- γ 2 gene adjacent to a serine phosphorylation site contributing to a phenotype with severe obesity, which is not associated with type 2 diabetes or hyperinsulinemia. In this study, we have investigated whether variants in the PPAR- γ 2 gene are associated with obesity and extreme insulin resistance in obese patients with impaired glucose tolerance (IGT), and asked whether altered levels of free fatty acids (FFA) might be associated with PPAR- γ 2 gene variants. The Pro115Gln, Pro12Ala, Pro467Leu, Val290Met and a silent polymorphism CAC478CAT were examined in 48 subjects with IGT and insulin resistance (IR), characterized by euglycemic hyperinsulinemic clamps, and in 52 healthy, lean, insulin sensitive (IS) controls. We found one patient in the IR group with the Pro115Gln variant. The body weight of this individual (BMI 28.5 kg/m²) was within the average of the IR group (30.3 \pm 0.8 kg/m²), but whole body glucose uptake (18 μ mol/kg per min) was lower as compared to the entire IR group (29 μ mol/kg per min) (p<0.05). The Pro12Ala variant was not associated with differences in BMI, in the degree of insulin resistance or in plasma FFA concentrations between the IR and IS group. The Pro467Leu, Val290Met mutations and the silent polymorphism CAC478CAT were not detected in any group. In conclusion, the Pro12Ala variant in the PPAR- γ 2 gene is common, but not associated with diabetes, whereas the Pro115Gln mutation is rare and may cause severe insulin resistance, although this was not the case for previously reported carriers of the Pro115Gln mutant.

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GENETIC MAPPING OF IRS-2 BY DETECTION OF SNPs AND HAPLOTYPES ASSOCIATED WITH INSULIN RESISTANCE.

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Background & aims: IRSs (insulin receptor substrates) are susceptibility genes for both insulin resistance and β -cell dysfunction in complex diseases such as non insulin-dependent diabetes mellitus (NIDDM) or polycystic ovary syndrome (PCOS). To understand heterogeneity at the gene level we investigated SNPs (single nucleotide polymorphisms) and complex haplotypes of IRS-2 gene in a Caucasian population (n = 53) with PCOS and variable insulin resistance (HOMA_{IR} 3.2 \pm 0.6). **Methods:** SNPs were searched by fluorescence-based sequencing of unphased DNA (ABI-373A) covering the coding region, 1106 bp 5' and 300 bp 3'UTR and regions at 4 kb upstream. Haplotypes were estimated from homozygous individuals, single-site heterozygous and then by inferential steps using Clark's algorithm, followed by assignment of haplotype pairs in each individual. **Results:** cSNPs were detected at sites 723, 816, 829, 1031, 1033, 1048, 1057 and a new SNP (C/T) at -769 site in 5'UTR. These variations and SNP 1157-21 and -22 upstream were arranged as 8 unambiguous and 22 unique haplotypes. The most frequent (47.2%) was haplotype 1 branched from the root in the phylogenetic tree by Gly1057Asp mutation. Mutation 1057 (G/A) was also found in two other branches partially explaining heterogeneity in analysis of this variant. Less frequent (around 20%) were haplotypes 2 (site 1033), 3 and 4 (sites -769, 723, 816, 829). In PCOS the most frequent haplotype pairs were 2/4 (18.4%) and 1/4 and 1/2 (10.3%) while all remaining pairs were < 5.1%. When population was stratified as function of HOMA_{IR} (cut off 2.1), insulin resistant and 33% of glucose intolerant patients were associated with pairs 1/4 (HOMA_{IR} 5.3 \pm 3.5) and 1/2 (HOMA_{IR} 2.5 \pm 0.3). Haplotype 3/4, 3/5 2/2 and 5/6 (HOMA_{IR} < 1.7) were found only in non-insulin resistant individuals and no haplotypes were associated with *acanthosis nigricans*. In conclusion, despite high heterogeneity of IRS-2 gene, the role in insulin resistance may be considerably unraveled by new strategies using dense genetic mapping based on SNPs and complex haplotypes.

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GENETIC POLYMORPHISM OF APOLOPOPROTEIN E ASSOCIATED TO TYPE 2 DIABETES MELLITUS IN A MEXICAN POPULATION.

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Background and Aims: Genetic and environmental risk factors are important to predisposition and development of type 2 diabetes mellitus (DM2), however, such risk factors could have population specific characteristics. In this study we analyzed the distribution of the genetic polymorphism of apolipoprotein E (apo E) and its association with lipid profile in patients with DM2 from the west of Mexico. **Materials and Methods:** Apo E genotypes including the three common allele (e2, e3, and e4) were identified by enzymatic amplification (PCR) of genomic DNA from blood samples and analysis of restriction profiles. Distribution of genotypes and alleles found in general population were compared with the usual distribution of apo E genotypes in patients with DM2. **Results:** In the general population (n=179) the allele that predominates is e3 with 83.8% followed by e4 (8.4%) and e2 (7.8%) respectively. Also a statistically significant relationship was found between high levels, of both total cholesterol and LDL with apo E 2/2, and apo E 3/4 genotypes (p<0.001 and 0.059). A similar increase of lipid profile was also observed with apo E 3/2 genotype, however such increase was not statistically significant. In the group of patients with DM2, the frequency of the allele e2 was higher (16.0%) than those found in the general population (7.8%). Such increase in the allele e2 was closely associated to DM2 (p<0.019). There was not association between lipid profile and genotypes in patients with DM2. **Conclusions:** This study show that the allele e2 is associated to DM2 in patients from the west of Mexico. Since e2 is also associated to clinical complication of DM2, such information could be considered for clinical follow up of the patient and prevention of DM2.

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A NEW MUTATION TNFR2 GENE ASSOCIATED TO HYPERTENSION IN CONTROL AND TYPE 2 DIABETIC PATIENTS.

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BACKGROUND AND AIMS: Association and linkage population studies have reported Tumor Necrosis Factor Receptor 2 (TNFR2) to be related with multiple metabolic disorders such as dyslipidemia, hypertension, obesity and insulin resistance in general population. TNFR2 exon 6 encodes a small portion of the transmembrane region and contains the position of the proteolytic cleavage site that produces the soluble form of TNFR2 (sTNFR2) through a mechanism named shedding.

TNFR2 exon 6 gene was studied in order to detect DNA variability which, possibly affecting receptor shedding, could be associated with susceptibility to type 2 diabetes mellitus or its metabolic disorders. **MATERIALS AND METHODS:** 119 type 2 diabetic patients and 67 matched subjects were studied. Mutations in the TNFR2 exon 6 gene were analyzed by means of automated sequencing of PCR products. The clinical parameters analyzed were blood pressure and body mass index (BMI). Leptin, sTNFR1, sTNFR2 and lipidic parameters were measured in all subjects. **RESULTS:** A previous reported polymorphism at position 196 (T>G) was identified. Moreover, a new mutation in intron 5 (C>T) unknown until date was described. All individuals homozygous for T allele in exon 6 were also homozygous for allele C in intron 5. No significant differences in allelic or genotypic frequencies between controls and patients were observed. Control and diabetic men carrying the homozygous haplotype for exon 6/intron 5 (TT/CC) showed significant lower levels of systolic blood pressure (SBP) (126.7 ± 9.6 vs 144.2 ± 18.7 mm Hg for controls and 135 ± 18.9 vs 152 ± 22.1 mm Hg for diabetic patients), but only those TT/CC diabetic men showed a trend towards lower levels of sTNFR2. A multiple linear regression model either SBP or DBP-TNFR2 haplotype controlled by group, age, sex, BMI, triglycerides, HDL-cholesterol, sTNFR1, sTNFR2 showed that both SBP and DBP levels were independently determined by the haplotype TT/CC (subjects carrying the haplotype are those with the lower blood pressure). **CONCLUSIONS:** These results suggest that this new genetic variation at TNFR2 gene may influence blood pressure levels independently of age, sex, BMI, lipidic parameters and sTNFRs in controls and type 2 diabetes.

This project was supported by FIS 00/0757 and Fundacio Marato de TV3(99/2010).

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TNF- α GENE POLYMORPHISM IS ASSOCIATED WITH INSULIN RESISTANCE IN THE SARDINIAN POPULATION.

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Background and Aim: TNF- α is a cytokine proposed to interfere with insulin signaling at the level of the insulin receptor. We evaluated the association of G \rightarrow A polymorphism at position -308 of the TNF- α gene promoter with insulin resistance measured by the Homeostasis Model Assessment (HOMA-IR). The G308A, being in the promoter region of the gene does not affect the amino acids sequence of TNF- α , but might affect the amount of protein translated interfering with insulin sensitivity. **Materials and Methods:** 702 individuals with type 2 diabetes and 518 non diabetic subjects, unrelated, comparable for age, sex, plasma lipids, and BMI were screened for the G308A variant. All subjects with fasting serum glucose <140 mg/dl were tested with OGTT, those with values >140mg/dl in two occasions were considered diabetics; fasting glucose and insulin were measured in all subjects. **Results:** In the non-diabetic group BMI, HOMA-IR and uric acid were significantly higher ($p<0.01$) in the hypertensive subjects as compared to normotensive. Only HOMA-IR was significantly higher ($p<0.05$) in normotensive non-diabetic with family history positive for hypertension as compared to those with negative family history. The same differences, although non significant, were present also in type 2. The A allele at position -308 was present in 4.9% type 2 and 7.3% non diabetics ($\chi^2=5.33$, $p=0.02$). No differences were present when the analysis was performed for BMI or hypertension, irrespective of diabetes status. HOMA-IR in A mutated subjects was significantly lower (4.8 ± 2.8 vs. 8.0 ± 6.7 , $p<0.05$) and associated with significantly lower circulating TNF- α [pg/ml, median and (range) 2.5, (0.15-120) vs. 10, (0.15-180), $p=0.0241$] as compared to G allele carriers. **Conclusion:** Our data, confirming the important role played by TNF- α on insulin sensitivity, are consistent with the hypothesis that in Sardinian population the G308A variant might be protective against the future development of type 2 diabetes in susceptible individuals, ameliorating insulin sensitivity.

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A TUMOUR NECROSIS FACTOR ALPHA GENE POLYMORPHISM INCREASES RISK OF INSULIN RESISTANCE AND THE METABOLIC SYNDROME IN OBESITY

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Background and Aims: Tumour necrosis factor alpha (TNF α) is a multifunctional cytokine that when produced by the adipose tissue, acts as an endocrine signal that may cause increased insulin resistance of the surrounding tissue. Secretion of TNF α is increased in the obese state, and could be the link between obesity and the development of Type II (non-insulin-dependent) diabetes. We investigated whether a polymorphism in the promoter region (-308G/A) of the TNF α gene was associated with increased risk of insulin resistance and dyslipidemia in an obese Australian population.

Materials and Methods: Obese, non-diabetic subjects (146 female, 34 male) were genotyped using PCR-RFLP techniques and anthropometric and biochemical measurements were compared. A homeostasis model assessment (HOMA) score was used to gauge the level of insulin resistance.

Results: Subjects homozygous for the mutation resulting in the A allele (7 %) had higher fasting insulin (226 ± 27 vs 131 ± 7 pmol/l, $p<0.001$), higher systolic blood pressure (143 ± 5 vs 129 ± 2 mmHg, $p<0.001$) and lower HDL cholesterol (1.13 ± 0.05 vs 1.25 ± 0.03 mmol/l, $p=0.04$) than subjects homozygous (59 %) for the G allele or subjects who were heterozygous (34 %). HDL cholesterol was negatively correlated to HOMA scores ($r = -0.710$, $p<0.001$) in subjects with the A allele only. Insulin resistance was associated with BMI and waist circumference in all subjects.

Conclusions: The -308 A/G variant conveys an increased risk for the development of insulin resistance in obese subjects. The presence of low HDL cholesterol further increases the risk for the development of insulin resistance, but only in those subjects carrying the A allele.

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PROMOTER POLYMORPHISM OF THE PARAOXONASE (PON1) GENE MAY BE INVOLVED IN INSULIN SENSITIVITY

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Background and Aims: Human serum paraoxonase (PON1) has been shown to reduce the susceptibility of LDL to lipid peroxidation. The objective of the study was to determine whether the -108C/T polymorphism of the PON1 gene, which is related to the serum PON1 concentration, is associated with insulin sensitivity. **Materials and Methods:** Seventy Japanese (20 normal subjects, 14 subjects with IGT and 36 type 2 diabetic patients) were recruited, and the euglycaemic-hyperinsulinaemic clamp study was performed to assess insulin sensitivity. Genomic DNA was extracted from whole blood, and the -108C/T polymorphism was detected by the direct sequencing method.

Results: The frequencies of -108CC, -108CT and -108TT genotypes were 32.9, 45.7, and 21.4%, respectively. There were no significant differences in frequencies of these genotypes between the 3 groups. Glucose infusion rate (GIR) was significantly higher in -108CC genotype than in -108CT or -108TT genotype (7.6 ± 3.2 , 5.6 ± 2.8 , 5.5 ± 2.4 mg/kg/min, respectively; $p=0.025$, ANOVA). The stepwise regression analysis for GIR revealed that the -108C/T polymorphism was a significant contributor ($F=13.082$), as well as BMI, fasting immunoreactive insulin and HbA_{1c} ($R^2=0.520$, $p<0.0001$). **Conclusions:** It has been reported that oxidative stress causes insulin resistance. This polymorphism may influence insulin sensitivity through modulating serum antioxidant capacity.

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Identification of genetic polymorphisms in the coding region of the hormone sensitive lipase gene and their impact on insulin sensitivity of lipolysis and glucose disposal

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Background and Aims: Free fatty acids released during triglyceride lipolysis play an important role in obesity-associated insulin resistance of glucose disposal. The individual sensitivity of lipolysis to the suppressive effect of insulin varies greatly among healthy subjects. The aim was to assess whether genetic variants in the hormone sensitive lipase (HSL) gene contributes to the biological variation of insulin sensitivity of lipolysis and glucose disposal.

Materials and Methods: We determined insulin sensitivity of lipolysis (suppression of isotopically [primed-continuous infusion of d5 glycerol] measured glycerol rate of appearance) and of glucose disposal using a three-step (N=20) or standard (N=53) hyperinsulinemic euglycemic clamp in 73 healthy, unrelated subjects. To assess the possible role of genetic polymorphisms, we sequenced the coding region of the HSL gene and the non-coding exon B from these subjects using overlapping single-strand conformational polymorphism analysis.

Results: We identified 2 silent mutations and 3 amino acid polymorphisms: Arg262Met (prevalence 5 %), Glu620Arg (31 %) and Ser681Ile (22 %). The latter two are located in the regulatory domain of HSL but neither one had a significant impact on insulin sensitivity of lipolysis or glucose disposal (with and without adjustment for obesity and age as covariates, all p values > 0.20).

Conclusions: We conclude that a number of genetic polymorphisms in HSL exist of which some are highly prevalent. It is unlikely, however, that these polymorphisms contribute to the biological variation of insulin sensitivity. Therefore, HSL does not appear to be a major candidate gene for insulin resistance or type 2 diabetes.

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ANALYSIS OF THE HUMAN HEXOKINASE II PROMOTER IN TRANSGENIC MICE: LACK OF FUNCTIONAL INSULIN RESPONSE UNIT WITHIN 4.0 KB

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Background and Aims: Hexokinase II (HKII) plays an important role in facilitating glucose uptake in response to insulin. HKII may have a role in the development of Type 2 diabetes and insulin resistance since the reduction in the levels of HKII mRNA and enzyme production has been observed in patients with Type 2 diabetes. The aim of this study was to delineate the molecular mechanisms of insulin induction by identifying the cis-acting DNA element(s) that mediate(s) the effect of insulin on HKII gene transcription. **Materials and Methods:** We have generated transgenic mouse lines harbouring either the 97, 254, 505, 819 or 4077 bp human HKII promoter driving the expression of a luciferase reporter gene. Basal luciferase activity was determined in tissue homogenates from 4 wk old transgenic mice using Wallace GeneLux kit. Insulin induction studies were performed on 5 wk old transgenic mice after 12 h fasting by repeated injections of D-glucose followed by analyses of endogenous HKII transcription and reporter gene expression. **Results:** Our results showed that the -505 bp promoter construct was the shortest one that was capable of driving expression of the reporter gene in a tissue-specific manner and giving any significant basal promoter activity (for all tissues, -254 vs. -505, p<0.01; -505 vs. -819, NS). Surprisingly, luciferase activities in transgenic mice carrying either -505 or -819 constructs were significantly higher than those of the -4077 construct (for all tissues, -505 vs. -4077, p<0.01; -819 vs. -4077, p<0.05). In response to glucose injections, no significant induction of luciferase activity was observed in the treated group compared to the control group with any of the promoter constructs used, even though the treatment resulted in a ~3-fold induction of endogenous HKII mRNA. **Conclusions:** Luciferase activities indicate that major promoter elements responsible for the tissue-specificity and basal activity of the human HKII gene expression are located within the -505 bp segment of the promoter. In addition, the presence of a powerful in vivo silencer element between -819 and -4077 bp was revealed. However, our results suggest a lack of functional insulin response unit in the 4077 bp region of the human HKII gene promoter in mice.

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CHARACTERISATION OF THE HUMAN SKELETAL MUSCLE GLYCOGEN SYNTHASE GENE (GYSI) PROMOTER

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Background and Aims: Impaired activation of skeletal muscle glycogen synthase (GS) by insulin is typical for type 2 diabetes and the *GYSI* *XbaI* polymorphism has been associated with type 2 diabetes and the metabolic syndrome. Our aim was to obtain information on the transcriptional regulation of the *GYSI* gene and to study whether variants in the promoter region could explain the previous genetic associations. **Materials and Methods:** Seven fragments of 121, 250, 544, 692, 995, 1707 and 2158bp of the *GYSI* promoter, all including 59 bp downstream from the transcription initiation site were cloned into a pGL3-Basic vector containing the firefly luciferase reporter gene and expressed in human kidney cells (HEK293), in mouse C2C12 myoblasts and in differentiated myotubes. The effect of a putative cAMP responsive element (CRE) located at nucleotides -227 to -235 was evaluated by forskolin stimulation (10µM for 24h). The region with highest promoter activity (-445 to -1013 bp) was screened for mutations by single-stranded conformational polymorphism (SSCP) in 20 sib pairs discordant for both the *XbaI* polymorphism and the metabolic syndrome. **Results:** In relation to the -250 fragment, the promoter activities of the different constructs were 0.08, 1.0, 0.8, 0.8, 0.5, 0.4 and 0.3 in HEK293; 0.4, 1.0, 1.3, 2.3, 1.0, 1.0 and 0.5 in myoblasts and 0.1, 1.0, 1.0, 3.7, 1.9, 1.4 and 1.0 in myotubes. The highest promoter activity was observed for the -692 bp fragment in C2C12 myotubes (3.7 ± 0.9 fold of the -250 bp fragment p=0.0003). Forskolin treatment resulted in a 30% decrease in activity of the -121, -250 and -692 bp fragments (p=0.019, p=0.0007 and p=0.0007, respectively) in myotubes, whereas insulin (100nM for 24h) did not have any significant effect. Mutation screening revealed new promoter variants and five pairs were genotype discordant. **Conclusions:** The -250 bp fragment is responsible for *GYSI* basal transcriptional activity in kidney cells, myoblasts and myotubes, whereas the myotube-specific expression is provided by the region between -544 and -692 bp. The *GYSI* promoter is sensitive to forskolin and contains several genetic variants.

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Functional significance of the UCSNP-43 polymorphism in the CAPN10 gene for proinsulin processing and insulin secretion in non-diabetic Germans

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Background and Aims: Recently, an association of the G allele in UCSNP-43 of calpain 10 with type 2 diabetes and decreased glucose disposal was reported. Calpain 10 is also expressed in pancreatic islets. The aim was to assess whether and how this polymorphism contributes to the biological variation of beta cell function.

Materials and Methods: We studied 73 non-diabetic subjects from the Southwest of Germany (G/G, N = 41; G/A, N = 29, A/A, N = 3) using a modified hyperglycemic clamp (10 mM glucose, added GLP-1, final arginine bolus). The genotype distribution was not different between NGT (N = 56) and IGT (N = 17) subjects (p = 0.74).

Results: First phase insulin secretion (adjusted for insulin sensitivity from hyperglycemic clamp and sex) was greater in G/G (2747 ± 297 pmol/min) than in G/A+A/A (1612 ± 156 pmol/min, p = 0.003). Insulin secretion in response to arginine (adjusted for insulin sensitivity) was also greater in G/G (9648 ± 1186 pmol/min) than in G/A+A/A (5686 ± 720 pmol/min, p = 0.04). The acute post-stimulus proinsulin:insulin (P/I) ratio was lower in G/G ($1.6 \pm 0.4\%$, 1st phase; 1.6 ± 0.2 arginine) than in G/A + A/A ($4.0 \pm 0.5\%$, 1st phase, p < 0.001; 2.5 ± 0.4 arginine, p = 0.03).

Conclusions: In conclusion, it appears unlikely that any association with type 2 diabetes of the UCSNP-43 polymorphism alone, i.e. irrespective of haplotype combinations, involves impairment of insulin secretion.

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THE IMPACT OF CALPAIN-10 GENE COMBINED-SNP VARIATIONS ON TYPE 2 DIABETES MELLITUS AND ITS RELATED METABOLIC TRAITS

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Background and Aims To investigate the impact of calpain-10 gene (CAPN-10) combined SNP variations on type 2 diabetes mellitus (T2DM) and its related clinical metabolic traits in Chinese. **Materials and Methods** 268 Chinese, 144 with NGT and 124 with T2DM. Plasma glucose (PG), insulin, c-peptide and free fatty acids (FFA) levels were measured at fasting and after oral glucose challenge. The insulin secretion and sensitivity were assessed by HOMA indices. CAPN-10 UCSNP44, UCSNP43, UCSNP19 and UCSNP63 were genotyped. **Results** (1). 14 genotype combinations of these four SNPs were observed in Chinese NGT subjects. 69% of the NGT population was composed of four genotype combinations, in the order of UCSNP44,-43,-19 and -63, i.e., combination A:TT-GG-DI-CC (haplotype combination 1121/1111) (frequency=10%), combination B:TT-GA-II-CC (1121/1221) (10%), combination C: TT-GG-II-CC (1121/1121) (26%) and combination D: TT-GG-DI-CT (1121/1112) (22%). (2). The frequencies of the SNP in single or in combinations were not differed significantly between NGT and T2DM groups. (3).The variation of clinical metabolic parameter levels shifted from completely normal towards glucose intolerance among genotype combination subgroups. In comparison between combination A to combination D, subjects in the former subgroups had higher PG levels with delayed peak after glucose challenge; less and lower decrement of FFA levels after challenge with no rising in late stage; more insulin secretion with delayed peak after challenge and the tendency of decreased insulin sensitivity. More than half of the variables remained statistically significant after adjusted with age, gender, body mass index and waist circumference. **Conclusions** The variation of calpain-10 gene has impact on the variations of clinical metabolic parameter related to type 2 diabetes mellitus. Such impact depends upon the haplotype as well as the haplotype combination.

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ALLELIC VARIATIONS IN CALPAIN 10, VITAMIN D RECEPTOR GENES AND SUSCEPTIBILITY TO TYPE 2 DIABETES IN A POLISH POPULATION.

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Introduction: The genes involved in the metabolism of vitamin D, for example vitamin D receptor (VDR), are considered to be candidates for type 2 diabetes (DM2). The evidence exists that calpain 10, a gene associated with DM2, may be regulated by vitamin D. **Aims:** 1) To search for the association of calpain 10 and VDR with DM2 in a Polish Population 2) To examine the interaction between calpain 10 and VDR. **Methods:** In total 200 individuals with DM2 were examined. The control normoglycemic group consisted of 113 individuals. The groups have been genotyped for calpain 10 SNP's 43, 19 and VDR Apal and BsmI variants so far. Genotyping for SNP63 is in process. SNP19 was examined by electrophoresis of the PCR product on agarose gel by size, while RFLP was used for the other markers. Since SNP's of Calpain 10 were in a very strong linkage disequilibrium, haplotypes could be assigned to phase-unknown individuals. Differences in distributions between the groups were examined by χ^2 test. The gene-gene interaction was assessed by multiple logistic regression. **Results:** The allele frequencies for DM2 patients and controls were as follows: Calpain 10: SNP43- G/A- 73,5%/26,5% vs. 71,0%/29,0%, SNP19- three 32 bp/two 32 bp repeats- 65,7%/34,3% vs. 62,2%/37,8%; VDR: BsmI- B/b- 35,0%/65,0% vs. 33,2%/66,8%, Apal- A/a- 48,0%/52,0% vs. 47,8%/52,2%, respectively. There was no difference between the groups when we analysed the allele, genotype and haplotype distribution in both genes. The multiple logistic regression analysis did not show an association, either. However in Calpain 10, G-three 32 bp repeats haplotype homozygotes (double SNP43 and SNP19 homozygotes) tend to be more frequent in DM2 (17,5%/9,6%, p=0,05). **Conclusion:** 1) The results suggest the association of Calpain 10 haplotype combinations with DM2 in a Polish Population. Adding SNP63 into the analysis will allow to analyse three locus haplotypes and to assign the attributable risk. 2) We did not find evidence for VDR association with DM2 or for calpain 10-VDR interaction.

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Polymorphism in the 5'-leader cistron of the beta-2 adrenergic receptor gene is associated to gynoid obesity in Italian population.

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Background and Aims: Obesity is a complex metabolic disorder with strong genetic components. Body fat stores and energy balance are largely controlled through subtypes of adrenergic receptors (Ars) coupled to guanine nucleotide binding proteins and adenylate cyclase. Some studies have reported associations of polymorphisms of both beta-2 and beta-3 adrenergic receptors (Ars) genes with obesity. The aims of our study is to assess the role of two polymorphisms in the beta-2 and beta-3 adrenergic receptors respectively in the development of obesity in Italian population.

Materials and Methods: We analysed beta-2 Gln27Glu and beta-3 Trp64Arg polymorphisms of adrenergic receptors gene in 295 obese subjects (body mass index ≥ 25 kg/m²) and in 126 control subjects (body mass index <25 kg/m²) matched for sex and age, using PCR-RFLP method.

Results: The frequency of Arg 64 phenotype is not statistically increased in obese compared to non obese subjects (8% vs 12% respectively). The frequency of Glu27 homozygous and of Glu27 phenotype is not statistically increased in obese compared with non obese subjects (10.5% vs 7.9% and 52% vs 47%). However the frequency of Glu27 homozygous is statistically increased in gynoid obese subjects compared to android obese subjects (21% vs 5,9% p=0.02, OR=3.1) and control subjects (21% vs 7,9% p=0.03).

Conclusions: Our results suggest that the beta-3 adrenergic receptor gene is not involved in the susceptibility to obesity and that the beta-2 Glu27 homozygous is implicated in gynoid obesity in Italian population

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CANDIDATE GENES IN COMPLEX DISEASES: ARE THEY USEFUL?
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Background and Aim: In complex diseases (type 2 diabetes, hypertension, obesity, etc) the candidate gene approach is used with the advantage to postulate a hypothesis, although a single gene cannot explain, by definition, the etiology of complex diseases. For this reason we decided to screen several candidate gene polymorphisms using a well matched case control population. **Materials and Methods:** Between 1994-2000 the SGGDS (Tonolo et al Diab. Nutr & Metab 1998) enrolled a total of 2451 subjects: 751 unrelated cases, 880 unrelated controls and 820 type 2 siblings of Sardinian origin from more than three generations. 65% were hypertensive, 40% were obese and 25% were microalbuminuric. Candidate genes were screened with the RFLP methods using published techniques validated in our laboratory. **Results:** *Trp64Arg* of the β -3 adrenergic receptor was associated with hypertension (Tonolo et al J Hypert 1999) and with increased diastolic blood pressure values to physiological noradrenaline infusion ($p < 0.01$); *C→A-188* of the Ob promoter gene was associated with $BMI > 30$ and significantly low plasma levels of leptin ($p < 0.05$); *G→A-308* of the TNF- α promoter gene with peripheral insulin sensibility and significantly lower levels of circulating TNF- α ($p = 0.024$); *ApoE* genotype 2/3 with significantly lower plasma levels of cholesterol ($p < 0.05$); *ACE II* although not significantly, was more frequent in micro-macroalbuminuric subjects; *Gly40Ser* of the glucagon receptor gene was not associated with diabetes or hypertension, but with a lower response to physiological glucagons infusion (Tonolo et al, Diabetologia 1997). **Conclusions:** Although no clustering of these polymorphism were evident from our data, and obviously no single polymorphism was causative of any of the considered complex diseases, nevertheless these approach could be used in the identification of intermediate phenotypes, closer to the gene product than the final phenotype and less modified by the environment and the genetic background, leading to a better phenotypic characterization of complex diseases as diabetes, hypertension, obesity etc.

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EFFECT OF THE CHOLESTERYL ESTER TRANSFER PROTEIN TAQIB GENE POLYMORPHISM ON PARAMETERS OF THE INSULIN RESISTANCE SYNDROME

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Background and Aims: The cholesteryl ester transfer protein (CETP) is responsible for the exchange of triglycerides and cholesteryl esters between lipoprotein particles leading to an increased hepatic clearance of HDL-cholesteryl esters. A high CETP activity reduces serum HDL, whereas subjects without CETP activity have high HDL levels. We investigated the association of the TaqIB CETP polymorphism and various parameters of the insulin resistance syndrome in a cross sectional population based study. **Materials and Methods:** We included 1029 persons without known cardiovascular disease or diabetes mellitus consecutively enrolled in the SAPHIR (Salzburg Atherosclerosis Prevention program in subjects with a High Individual Risk). Numerous clinical and laboratory parameters were determined. Insulin sensitivity was measured by a short insulin tolerance test. The TaqIB CETP polymorphism was determined by PCR, TaqI restriction and electrophoresis. **Results:** 35.2% were homozygous for the presence (B1B1), 46.7% heterozygous (B1B2), and 18.1% homozygous for the absence (B2B2) of the restriction site. HDL-cholesterol was significantly lower, triglycerides, apolipoprotein A and frequency of small dense LDL (sdLDL) higher in B1B1 compared to B2B1 and B2B2 subjects. In women we found a significant interaction effect between CETP genotype and obesity on HDL cholesterol. B1B1 women with a BMI and a waist circumference above the median had a 0.25 mmol/l lower HDL than B1B2 and a 0.23 mmol/l lower HDL than B2B2 women ($p < 0.001$). In men no interaction effect but a marked genotype to HDL correlation was found. Furthermore a high CETP effect on LDL size was detected in men ($p = 0.001$). B1B1 men had sdLDL in 36%, B1B2 in 24.6%, and B2B2 in only 14.5%. LDL-pattern B was found twice as frequent in males with obesity and insulin resistance as in insulin sensitive men. In **Conclusion** we found a significant sex specific effect of the TaqIB CETP polymorphism on the insulin resistance parameters HDL-cholesterol and LDL size.

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Variations in the WFS1 (Wolfram syndrome) gene are not associated with type 2 diabetes

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Background and Aims: Wolfram syndrome is a monogenic form of diabetes characterised by recessive inheritance, onset in childhood, and beta cell death. It is also known as DIDMOAD (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, Deafness). Obligate carriers of the Wolfram gene WFS1 have an increased frequency of diabetes. WFS1 variants have been associated with type 1 and type 2 diabetes, but this may reflect population stratification. We aimed to assess the contribution of WFS1 gene variants to the genetic heterogeneity of type 2 diabetes.

Materials and Methods: We screened 30 patients with type 2 diabetes for variants in the WFS1 gene by direct sequencing of genomic DNA. We used family based association methods in 155 parent offspring trios from the Diabetes UK-Warren Trios Repository, each ascertained via a Europid proband with type 2 diabetes.

Results: We selected 2 missense variants (R456H, R611H) and 3 single nucleotide polymorphisms (S855S, K774K, F341F), which were present in our screening panel with an allele frequency between 5.2-39.6%. There was no evidence for preferential transmission of the WFS1 variants from heterozygous parents to their type 2 diabetes offspring.

Conclusions: We conclude that there is no significant association between type 2 diabetes and these variations in the WFS1 gene. WFS1 is unlikely to be a major contributor to the genetic heterogeneity of type 2 diabetes.

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ASSOCIATION OF A HUMAN G-PROTEIN β 3 SUBUNIT POLYMORPHISM WITH REDUCED INSULIN SENSITIVITY.

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Background and Aims: The C825T polymorphism (exon 10) of the β 3 subunit of heterodimeric G-proteins (GNB3) is associated with hypertension and obesity. These known associations suggest a possible central association with insulin resistance. However, no such investigations are available yet. Aim of the present study was to investigate the possible contribution of this polymorphism to insulin sensitivity in a population based sample.

Patients and Methods: 197 consecutive subjects from a population based, prospective atherosclerosis and metabolism survey (SAPHIR) (age 54.5 ± 5.3 years, 30.5 % males) were genotyped using RFLP. Insulin sensitivity was measured by short insulin tolerance test (expressed as K_{ITT} mg/dl/min, available for 189 subjects).

Results: Genotypes were found in Hardy-Weinberg-equilibrium, a homozygous TT polymorphism was observed in 15 subjects (CC: 95; CT: 87). In the entire population K_{ITT} was 4.23 ± 1.29 mg/dl/min. No significant differences regarding K_{ITT} were observed between genotypes. However, in subjects with a $K_{ITT} < 4$, indicating reduced insulin sensitivity the TT genotype was observed in 10/87 subjects (11.5%) compared to 4/102 (3.9%) subjects with $K_{ITT} > 4$. The respective odds ratio for reduced insulin sensitivity in TT carriers was 3.18 ($p < 0.08$).

Conclusions: Our data, although of borderline statistical significance, suggest that the C825T polymorphism of the β 3 subunit of human heterodimeric G-proteins is associated with the risk of reduced insulin sensitivity in the general population.

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Type I Diabetes: Epidemiology

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Ethnic Minority Recruitment Into the Diabetes Prevention Trial, Type 1 (DPT-1)
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Background and Aims: The DPT-1 is a nation-wide study to prevent Type-1 diabetes using insulin as an immunomodulator for family members of type-1 diabetic patients. The National Institute of Health mandates recruiting of ethnic minorities into clinical trials, including Hispanics, African Americans, Asians and others. However, recruitment is difficult because of culturally-sensitive issues and lower rates of type-1 diabetes in these minority populations. The aim of the study was to enhance participation of family members of type 1 diabetics by involving minority community leaders and organizations.

Materials and Methods: Methods used to increase minority participation in the DPT-1 included screening of family members at family reunions, home visitations, health fairs at churches and schools. The objective of these community outreach efforts was (1) to screen for DPT-1 in minorities families with type-1 diabetes, (2) to establish awareness of the disease, and (3) to provide diabetes education.

Results: Of the total screened population from 1994-2000 at the Los Angeles County-University of Southern California Medical Center, there was a significant increase in screening during the period of 1997-2000, as compared to the years 1994-1996 (average of 1651 vs. 1060, subjects/year, $p < 0.01$).

Conclusions: Using culturally sensitive methods, ethnic minorities were more easily approachable and willing to participate in the DPT-1. Screening of subjects was significantly improved by community outreach activities.

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DIABETES IN CHILDREN OF SOUTH ASIAN ORIGIN IN WEST YORKSHIRE. AN UNUSUAL CASE MIX

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Background and Aims: The aim of this study was to define the type of diabetes present in children of South Asian origin in a Paediatric clinic in Bradford. The reasons for looking at this group of children is that, type I diabetes is uncommon in Pakistan, and that type II diabetes may be an emerging problem in this population.

Materials and Methods: We examined the case notes of the total clinic population, 140 children, and identified 55 children of South Asian origin.

Results: The majority of children were born in Bradford, but 42 of the families originated from Pakistan, 11 from India, 1 from Bangladesh and 1 from Saudi Arabia. These children account for 39% of the diabetic population, compared to 26.5% of the primary school population, and 31.5% of the secondary school population. We found a wide spectrum of diabetic diseases within the Asian children compared to the Caucasian children in the clinic. The following clinical groups were identified.

- a) 32 children with type I diabetes had no other affected close relatives.
- b) 12 children with type I diabetes had other affected family members.
- c) 1 child presented with type II diabetes.
- d) 2 children presented with MODY type diabetes.
- e) 8 children with type I diabetes had other genetic abnormalities.

Conclusions: 20% of the Asian diabetic children had unusual types of diabetes not seen in the Caucasian children.

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INCIDENCE OF TYPE 1 DIABETES MELLITUS OVER EIGHT CONSECUTIVE YEARS AMONG 15-39-YEAR AGED LITHUANIAN POPULATION

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Background and Aims: To document the incidence of Type 1 (insulin-dependent) diabetes mellitus among Lithuanian 15-39 - year - aged population from 1991 to 1998.

Materials and Methods: A specifically developed contact system with all endocrinologists/diabetologists and general practitioners involved in the diabetes care covering 100% of the 15-39-year - aged Lithuanian population was the initial data source. Annual reports from regional endocrinologist's /diabetologists, statistical note-marks of diabetic patients who visited Medical Units, death certificates and patients lists from Diabetes Societies remained as secondary independent sources for case ascertainment.

Results: The total of 907 new cases (593 males and 314 females) of Type 1 (insulin-dependent) diabetes mellitus were recorded among the 15-39-year-aged population during the period 1 January 1991 - 31 December 1998. The cumulative incidence density per year was 8.07/100,000 (95% Poisson distribution confidence interval 7.56-8.62) and was slightly higher among males (10.46/100,000, 95%CI 9.65-11.34) than among females (5.64/100,000, 95%CI 5.05-6.03), $p < 0.0001$. Age standardized overall incidence rates for males and females were 10.47 and 5.65, respectively. Male/female ratio was 1.85. Results of the linear regression models showed that the incidence density of insulin-dependent diabetes mellitus in 15-39-year age group had the tendency to increase.

Conclusions: The results suggest that the incidence data of Type 1 diabetes mellitus in Lithuania is appropriate to those in Poland and lower than in other countries of Baltic Sea region. The data contributes to the knowledge of the incidence of Type 1 diabetes mellitus in Eastern Baltic countries, an area that until now has been lacking epidemiological data on diabetes among 15-39 - year - aged population.

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ROMANIAN DIABETES EPIDEMICS PROGRAMME ("EPIDIAB") FIRST YEAR RESULTS

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Background. The burden of diabetes, mainly type 2, affects the health care system through both high costs and number of professionals involved, and high incidence. **Primary objectives of EPIDIAB P:** (1) epidemiological analysis of newly diagnosed diabetes (NDD), (2) quality of care assessment, (3) problems recording, (4) strategic prediction. As secondary objective, all data will be used to develop short, medium and long term new strategies, in order to improve the diabetes care. **Method.** The EPIDIAB study is designed to last 5 years, beginning with January 2000; it includes 12 counties covering one third of total population of Romania. Following data have been recorded: type of diabetes, demographic, screening and prevalence of complications, treatment, costs. **First year results.** In 2000, 15,156 persons have been diagnosed with diabetes: 6.4% Type 1 and 91.3% Type 2. The estimated incidence of Type 1 diabetes in our country is 14 /100,000 and for Type 2 is 200 /100,000. Estimated total number of NDD in Romania in 2000 is 44,000. Screening for complications was done in: 74.9% for retinopathy, 60.8% for nephropathy, 71.5% for neuropathy, 70% for dyslipidemia. Already present complications are: hypertension (45.3%), coronary heart disease (32%), retinopathy (12% from those investigated), nephropathy (7.2%), neuropathy (24.3%), dyslipidemia (48.8 %). Therapeutic structure is: diet alone - 26.3%, sulfonylureas - 30%, metformin 19.3 %, combined oral therapy - 13.8%, insulin 5.4 %. **Discussion.** The high prevalence of complications demands an early diagnosis through an active screening for diabetes. Primary health care should be developed and more involved and financial resources should be directed towards this field. Prevention should be developed in population at risk. Already diagnosed, more efforts should be addressed to screen for complications. Most of the patients are on medication, which means high cost from the beginning. **Conclusion.** After one year, EPIDIAB Programme demonstrates its utility in developing, strategies to improve the quality of diabetes care, at local and national levels.

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INCIDENCE OF TYPE 1 DIABETES MELLITUS IN THE OLSZTYN REGION, POLAND IN PATIENTS AGED 0-29 1994-2000

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Type 1 diabetes mellitus develops predominantly in childhood and adolescence. In many populations, type 1 diabetes mellitus represents a major health problem for patients and society because it leads to disability through complications and causes a risk of premature death. Currently, the epidemiology studies of type 1 diabetes mellitus have been conducted in highly industrialised urban areas. Varmia and Mazury, with the population of 778,000, is situated in the north – east of Poland and has a typical agricultural character. The first standardised register of type 1 diabetes mellitus for population aged 0-29 was established in the Varmia and Mazury region in 1993. **Aim:** To estimate the incidence of type 1 diabetes mellitus in the Varmia and Mazury Region, in the years 1994-2000. **Subjects and methods:** Subjects and methods: A registry was established in 1993 to collect new type 1 diabetes mellitus cases among people 0-29 years of age and was carried out, from 1994-2000. Data were collected according to the Diabetes Epidemiology Research International Group Recommendations. Data were analysed by the capture – recapture methods. The completeness of case ascertainment was estimated at 99%. **Results:** Between 1994 and 2000, in Olsztyn Region there were registered 234 new cases of Type 1 diabetes mellitus in the group aged 0-29 (118 males, 116 females). In the whole study group a significantly rising tendency in the incidence rate was observed (from 4.29 in 1994 to 11.75/100,000 in 2000), with the highest incidence rate for females aged 25-29 being 23.35/100,000 in 2000. A markedly higher incidence rate was observed in patients from urban areas. The greatest number of new Type 1 diabetes mellitus cases was found in autumn and spring in age group 0-14 and in autumn and winter in age group 15-29. **Conclusions:** The study documents the high incidence rate of Type 1 diabetes mellitus among people aged 0-29 in Olsztyn Region, Poland.

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THE INCIDENCE OF TYPE 1 DIABETES MELLITUS IN THE AGE GROUP 0-14 YEARS IN THE REGION OF WIELKOPOLSKA

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Background and Aims: The aim of the study was to determine the incidence of type 1 diabetes mellitus among children and adolescents in the region of Wielkopolska. The study group comprise a population of 0-14 year olds from 8 former provinces of western Poland. The area over which the data were collected constituted ca 50,000 km², i.e. 16% of total Poland's area. The population of children over the area was approximately 1,150,000 aged 0-14 years.

Materials and Methods: The data were collected using a questionnaire. A computer base was created to enable the accumulation and processing of data. Epidemiological data included age, sex, time of the onset of diabetes, appearance and character of clinical symptoms and family history. The final diagnosis of the disease was made after hospitalization. Three sources of data were collected. The incidence was expressed using the incidence coefficient, i.e. the number of newly detected cases of type 1 diabetes per annum per 100,000 children preselected age, separately for boys and girls, with respect to age: (0-4), (5-9), (10-14) years.

Results: The study made during 1998-2000. Over that period 292 cases of newly diagnosed type 1 diabetes were detected. There was no direct link between sex and the incidence of type 1 diabetes. The study group comprised 137(46%) boys and 155(53%) girls. The smallest number of cases was found in the (0-4)yr. group: 53(18.2%). The number of cases in the other groups was comparable: (5-9yr) - 116(39.7%); (10-14yr) - 123(42.1%) respectively. The incidence of insulin dependent diabetes mellitus over the period under study was 8.6/100,000/yr. The lowest number of new cases was in children aged 0-4 years (5.7 per 100,000), the highest i.e., almost twice as many in children 5-9 years of age (10.2 per 100,000). A slightly higher incidence was found in girls than in boys: 9.3 vs. 7.8. Children from urban areas were more susceptible than those from rural areas (urban-15.2; rural-8.2). Peaks of incidence were observed during autumn and winter seasons.

Conclusions: In 1998-2000 no significant increase in the incidence of type 1 diabetes was observed in the region of Wielkopolska. However, there were significant differences in the incidence rates in the relation to the particular age groups. The annual and regional incidence differences may be caused by environmental factors.

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As the overall risk of Type 1 diabetes in children increases age at diagnosis decreases.

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Background and Aims: The rising incidence of Type 1 diabetes in childhood is well documented. In particular, a steep rise in children under 5 years of age has been observed in some populations but not in Yorkshire. Set against this background of rising risk we investigated whether the overall age at diagnosis has decreased over the last 2 decades.

Materials and Methods: The population-based Yorkshire Children's Diabetes register recorded 2363 children (0-14 years) diagnosed with Type 1 diabetes between 1978 and 1998. Subjects are ascertained from independent sources and the register is 99% complete. Age-specific incidence rates were calculated for single year age-groups and linear regression was used to model change in cumulative risk and age at onset over time.

Results: The cumulative risk of a child developing Type 1 diabetes in this population significantly increased from 1 in 564 in 1978 to 1 in 334 in 1998 (linear regression coefficient -11.54, $p < 0.001$). Mean age at onset significantly reduced from a fitted mean of 9.2 years in 1978 to 8.6 years in 1998 (coefficient -0.029; $p < 0.001$). During the same period, mean age at onset significantly decreased from 9.3 to 8.8 years in boys (-0.025; $p < 0.001$) and from 9.1 to 8.4 years in girls (-0.032; $p < 0.001$).

Conclusions: Without imposing arbitrary age groupings or calendar period on the data we have shown that over 21 years in a population where incidence is rising at all ages, the mean age at onset has significantly decreased.

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Gender and Type 1 Diabetes in New Zealand Children and Adolescents

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Background and Aims: Canterbury is ranked internationally in the highest 10% of the incidence distribution for risk of childhood diabetes. In type 1 diabetes there is evidence for an excess of male presentations, a departure from the strong female sex bias in most autoimmune diseases. In addition, transmission of type 1 diabetes to offspring occurs more frequently from fathers than mothers. This study investigates these findings in a graphically defined thirty-year cohort of type 1 diabetes cases.

Materials and Methods: Ascertainment of type 1 diabetes in Canterbury individuals aged less than 20 years at diagnosis is complete for the years 1970-1999. Prior to 1982 incident cases were ascertained retrospectively from hospital records. Prospective ascertainment of all type 1 diabetes cases commenced in this region in 1982. Primary ascertainment of incidence cases was through notification from the attending diabetes physician or paediatrician. All new cases of type 1 diabetes were either admitted to the regional hospital or attended acute intervention outpatient clinics at the same institution.

Results: The total number of incident cases for the years 1970-1999 was 474 (256 males, 218 females). Incidence ranged from 2.40 cases/100 000 person years in 1970, to 26.59 cases/100 000 person years in 1998. The increase in incidence based on linear regression of these data is 0.59 cases/100 000 per year or an annual increase of 5% derived from regression of the natural logarithms of the incidence data. Between 1970-1999, the ratio of male to female cases was very similar before puberty. A male excess exists in cases presenting between 13 and 19 years of age. Examining the years 1990-1999 (n=210 cases), a male excess is observed from as early as 5 years of age, and becomes more marked with increasing age. A family history of type 1 diabetes was reported for 18 cases, including 12 individuals who had a parent with type 1 diabetes. Since 1990, blood samples have been taken at diagnosis for determination of antibodies against islet antigens and for characterisation of HLA-DQ alleles. Alleles of the HLA-DQ locus conferring protection against type 1 diabetes were more prevalent in females than in males in our cohort, but there was no evidence for a gender effect on the development of autoimmunity.

Conclusions: The relationship between gender and susceptibility to type 1 diabetes is complex, but these results encourage speculation on the underlying mechanisms.

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FIVE-YEAR INCIDENCE REGISTRATION OF TYPE 1 DIABETES IN CHILDHOOD IN NORTH RHINE-WESTPHALIA, GERMANY

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Background and Aims: Incidence estimates of Type 1 diabetes are subject to considerable random variation when based on small risk populations. Thus analysis of time trends is complicated. The aim of the present study was to estimate incidence rates and time trends of Type 1 diabetes in children 0-14 years of age during 1996-2000 based on the large risk population of North Rhine-Westphalia, the federal state of Germany with the largest population. The average risk population amounted to 2.93 millions children, corresponding to about one fourth of all children in this age group in Germany.

Materials and Methods: Newly diagnosed cases were prospectively registered by an active surveillance system (ESPED: Erhebungseinheit für seltene pädiatrische Erkrankungen in Deutschland) monthly contacting all pediatric and diabetes-specialized internal departments in the study region. A yearly retrospective inquiry among pediatric, internal, and general medical practices was used as secondary data source. Completeness of ascertainment was estimated by the capture-recapture-method. Point and interval estimates (95%-CI) of incidence rates (per 100,000 person-years) were based on Poisson distribution. Time trends were assessed by Poisson regression analysis.

Results: A total of 2,251 newly diagnosed diabetic children aged 0-14 years (1153 boys, 1098 girls) were registered during 1996-2000. Completeness of ascertainment was estimated as 89.2% (95%-CI: 87.5-91.1). The overall incidence rate was 15.3 (14.7-16.0). There was no difference in incidence between boys and girls ($p=0.972$). The incidence increased significantly with age ($p<0.01$), the estimates for the age groups 0-4, 5-9, and 10-14 were 10.2 (9.3-11.1), 16.5 (15.4-17.6), and 19.2 (18.0-20.4), respectively. The annual incidence rates ranged between 13.9 (in 1997) and 17.7 (in 2000). Over the five-year period, a significant annual incidence increase of 6.5% (3.4-9.7) was estimated.

Conclusions: To our knowledge, the present incidence registry is the largest one in Europe. The incidence rate of Type 1 diabetes in North Rhine-Westphalia is close to rates in the UK and Denmark. The striking incidence increase ranges high compared with trends in other European regions. If the incidence trend will continue, Type 1 diabetes would become one of the most common chronic diseases in childhood in Germany. That would have important implications on public health aspects of childhood diabetes care.

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THE INCIDENCE OF INSULIN-DEPENDENT DIABETES HAS NOT INCREASED IN THE 0-34 YEARS GROUP IN SWEDEN 1983-1998

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Background and aims: The incidence of childhood diabetes has increased in Sweden, and in many other countries. However, little is known about the time trend in a broader age span. We analysed the changes of insulin-dependent diabetes incidence in the 0-34 years group in Sweden 1983 to 1998.

Materials and Methods: The incidence and cumulative incidence rate per 100,000 and Poisson regression analysis of age-period effects was performed in a large data set of 11,751 cases, obtained from two Swedish nation-wide prospective diabetes registers.

Results: The incidence was 21.4 in 0-34 years old men and 17.1 in women. In the pre-pubertal boys (1-14 years) and girls (1-12 years) incidence increased over time, but it tended to decrease in the older age groups, especially in men. Average cumulative incidence rate at 35 years age was 748 in men and 598 in women. The cumulative rate in men was rather stable during four 4-year periods (736; 732; 761; 756), while in women it varied more (592; 542; 617; 631). In Poisson regression analysis the incidence in 0-34 years old men did not vary between the 4-year periods ($p=0.63$), but time changes among the 3-year age groups differed ($p<0.001$). In females the incidence between the periods varied ($p<0.001$), being significantly lower in 1987-90 compared to 1983-86, but time changes in the age groups did not differ ($p=0.08$). For both sexes median age at onset in 1995-98 was lower than in 1983-86 ($p<0.001$) (15.0 and 12.5 years in males; 11.9 and 10.4 in females, respectively).

Conclusions: During a 16-year period the incidence of insulin-dependent diabetes did not increase in the 0-34 years age group in Sweden, while median age at onset decreased. A shift to younger age at onset seems to explain the increase of the disease seen in childhood.

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Type 1 Diabetes – Mechanisms

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VITAMIN D RECEPTOR mRNA AND VDR PROTEIN LEVELS IN RELATION TO VITAMIN D STATUS AND TO VDR GENOTYPE

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Background and aims: We, and others, have demonstrated associations between the polymorphism of the vitamin D receptor (VDR) gene and Type 1 diabetes. Furthermore, associations have also been found with insulin secretion and components of the insulin resistance syndrome. However, the VDR polymorphisms have no known functional significance and therefore could either implicate the VDR gene or a closely linked locus. We have investigated whether VDR polymorphisms mark differences in VDR mRNA or protein levels.

Materials and Methods: PBMCs were isolated from 41 Bangladeshi subjects, typed for VDR FokI, BsmI, ApaI and TaqI polymorphisms, and used for mRNA and VDR protein studies. Total VDR mRNA was quantitated using a TaqMan and VDR protein by an immunoassay. Stepwise multiple regression models [$p<0.05$] were used for statistical analysis.

Results: For VDR mRNA the 'best-fit' model ($p = 0.004$) showed FokI [$p = 0.044$] and TaqI [$p = 0.04$] genotypes and insulin secretion index [$p = 0.042$] to be determinants. For VDR protein levels the 'best-fit' model ($p = 0.006$) showed TaqI genotype [$p = 0.005$] and current serum dihydroxyD levels [$p = 0.03$] to be determinants. The TaqI tt genotype was associated with the lowest VDR mRNA and protein levels. VDR mRNA copy number did not appear as a main determinant of VDR protein concentration (p value was 0.06).

Conclusions: these studies demonstrate that VDR polymorphism is a determinant of the amount of VDR mRNA and protein expressed and therefore give biological credibility to the previously described associations with the VDR gene.

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SUPPLEMENTATION OF VITAMIN D IN EARLY CHILDHOOD COULD PROTECT AGAINST TYPE 1 DIABETES MELLITUS

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Background and Aims: Exposure to some environmental factors in early childhood may predispose or protect against insulin-dependent diabetes mellitus in later life. Supplementation of vitamin D in the early childhood may play some protective role against the development of insulin-dependent diabetes. Therefore, the aim of our study was to investigate the presence of association between intake of vitamin D during the early childhood and the development of type 1 diabetes later.

Materials and Methods: We studied 150 patients with type 1 diabetes mellitus with the onset of the disease at the age younger than 15 years old and 50 age-matched randomly selected control subjects. The enrolled subjects were asked to fill out the proposed questionnaire which included information regarding the vitamin D supplementation in the early childhood, its duration, type of nutrition during the first year of the life, perinatal events, vaccinations provided and characteristics of early growth. The statistical analysis was performed using Mantel-Haenszel method and logistic regression analysis.

Results: Supplementation of vitamin D in early childhood provided some protection against development of type 1 diabetes mellitus in children with odds ratio (OR) 0.69 (CI 0.57-0.87) compared to those children which did not have vitamin D supplementation. OR for protective effect of vitamin D was 0.83 (CI-0.57-1.47) for children younger than 5 years old, 0.81 (CI-0.57-1.27) for those 5-9 years old and 0.49 (CI-0.37-0.69) for subjects aged 10-14 years old. There was no any significant influence of shortness of breast feeding (less than 3 months), maternal age over 35 years old, low birth weight (less than 2500 g) on the preventive efficacy of vitamin D. Children receiving vitamin D for less or longer than 1 year exhibited the similar risk reduction (OR - 0.67 and 0.61, respectively).

Conclusions: Vitamin D given in the early childhood may protect against the development of insulin-dependent diabetes mellitus during the later periods of life. The preventive significance and possible mechanisms of revealed association require further studies.

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CYCLO-OXYGENASE EXPRESSION IN IDENTICAL TWINS DISCORDANT FOR TYPE 1 DIABETES

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Background and Aims: Cyclo-oxygenase-2 (COX-2) is responsible for the synthesis of prostaglandin in mammalian cells. A recent report suggests an aberrant monocyte COX-2 expression in type 1 diabetes, and those at risk of the disease. Aberrant monocyte COX-2 expression may result in antigen presenting cell dysfunction, thereby predisposing these individuals to diabetes. To define the nature of monocyte COX-2 expression in type 1 diabetes and to determine whether increased monocyte COX-2 expression could be inherited, we are studying a large cohort of identical twins discordant for type 1 diabetes.

Materials and Methods: Using human peripheral blood mononuclear cells (PBMCs) we isolated CD14⁺ monocytes and tested for COX-2 messenger RNA using quantitative RT-PCR, intracellular COX-2 protein expression by FACS and on western blot (WB), as well as functional studies employing cytokine and prostaglandin secretion, pre and post lipopolysaccharide (LPS) stimulation with and without COX-2 inhibitors.

Results: Normal healthy controls do have detectable COX-2 mRNA but protein expression was below detectable levels on FACS and on WB. In contrast, twins with type 1 diabetes have COX-2 protein expression on both FACS and WB. Non-diabetic twins, at low disease risk with normal glucose tolerance and without diabetes-associated autoantibodies showed COX-2 expression in unstimulated cells intracellularly on FACS compared to normal controls ($p < 0.004$). Monocyte response to LPS is abnormal in type 1 diabetes, the maximum response was greater in controls than in diabetics (WB and FACS, $p < 0.003$ and $p < 0.03$) but also higher in cells from non-diabetic twins than from their diabetic twins (WB, $p < 0.02$).

Conclusions: Our study confirms that basal COX-2 expression is abnormal in type 1 diabetes. Monocytes in type 1 diabetes are activated and this activation is not entirely due to diabetes but associated with genetic susceptibility to disease. Whilst activated monocytes could predispose an individual to type 1 diabetes, our results show that such activation is not predictive of the disease.

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INCREASED MONOCYTE CYCLOXYGENASE-2 EXPRESSION IN FEMALE NON OBESE DIABETIC MICE

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Background and Aims: Non-obese diabetic (NOD) mice are a spontaneous model of type 1 diabetes mellitus. Diabetes incidence in our colony is 55% in females, but only 15% in males, at 30 weeks of age. Cyclooxygenase (COX), a key enzyme in prostanoid metabolism, is expressed in two isoforms, constitutive (COX-1) responsible for physiological processes and inducible (COX-2) responsible for inflammatory prostanoid synthesis. Aberrant COX-2 expression is present in man and may result in an antigen presenting cell dysfunction, thereby predisposing to diabetes. The aim of this study was to determine expression of COX-2 in NOD mice and its relationship with diabetes incidence.

Materials and Methods: CD11b⁺ monocytes were isolated from splenocytes of diabetic female, non-diabetic female and male NOD mice (range 4-25 weeks of age), as well as control strains and examined for COX-1 and COX-2 messenger RNA using conventional RT-PCR. We also tested for protein expression using Western blotting and carried out functional studies for cytokine and prostanoid secretion. All tests were carried out before and after lipopolysaccharide (LPS) stimulation.

Results: Basal COX-2 message expression in non-diabetic female mice was greater than males (50 vs. 0%, $p < 0.025$), but there was no difference between diabetic and non-diabetic female NOD mice (60 vs. 50%, $p < 0.3$). There was no difference in COX-1 expression between the groups. Western blot confirmed detectable levels of COX in diabetic and non-diabetic female NOD mice.

Conclusions: The expression of COX-2 in 50% of non-diabetic female mice but not in males corresponds well with the NOD/Ba colony incidence of diabetes and suggests COX-2 may be associated with the development of diabetes in this model.

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FUSIDIC ACID REDUCES INCIDENCE OF DIABETES IN BB RATS.

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Aim: Fusidic acid and its sodium salt (fusidin) are anti-staphylococcal drugs which possesses immunomodulatory properties. The aim was to investigate whether high concentrations of fusidin could lower the diabetes incidence in BB rats, and to measure the activity of fusidin in various organs.

Methods: Three groups of BB rats were used: 63 rats received fusidin dissolved in drinking water; 65 rats received chow containing fusidin; and 32 rats served as controls. The animals were placed randomly in the groups after weaning at 3 weeks of age, and were sacrificed at diagnosis of diabetes or at 200 days. The content of fusidin in the organs were examined microbiologically.

Results: The incidence of diabetes was 50.8% ($p = 0.01$) for animals receiving fusidin in the drinking water, and 56.9% ($p = 0.01$) for fusidin chow treated rats, compared to 68.1% for the control group. The incidence was lower for male than female rats in both experimental groups while no gender difference was seen in the control group. The daily intake of fusidin in the chow group was 339 mg/day for male and 245 mg/day for female rats. The corresponding values in the fusidin drinking water group were 200 and 163 mg/day, respectively. However, the female rats had a substantially higher content of fusidin in their organs than the males regardless of the administration way and regardless of diabetes outbreak or not; thus, the amount of fusidin in pancreas for the non-diabetic animals receiving fusidin chow was 10.7 ± 3.1 vs 1.7 ± 0.3 μg fusidin/g wet weight for female respective male rats. The weights of the non-diabetic animals in the three groups were similar. Interestingly, the fusidin treated non-diabetic rats displayed a lower random blood glucose level than the controls (7.3 ± 0.2 vs 8.6 ± 0.4 mmol/l, $p = 0.002$).

Conclusions: Fusidin is well absorbed after oral administration and it reduces significantly the diabetes incidence in BB rats. Fusidin accumulates substantially more in female rats, which may be due to the steroid structure of fusidin. Treatment with fusidin lowers random blood glucose in non-diabetic BB rats.

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EFFECTS OF SODIUM VANADATE IN PREDIABETIC BB-DP RATS

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Background and Aims: This study evaluated the effects of sodium vanadate on diabetogenesis process in BB-DP rats. **Materials and Methods:** We used 96 BB-DP rats of prediabetogenic age (40-50 days), divided into 4 equal groups (sex, age, weight). Groups V1, V2, V3 were treated with different doses of sodium vanadate (0.1, 0.2 and 0.3 mg/ml respectively) and sodium chloride (0.5 mg/ml) in drinking water for 7 days. Group C, the untreated control, received only sodium chloride (0.5 mg/ml) in drinking water for 7 days. The animals were allowed to drink the solution *ad libitum*. Blood glucose (BG), glycosuria, ketonuria and body weight (BW) were determined before starting, daily in week 1, and once a week for 12 weeks. **Results:** The incidence of diabetes was lower in the rats treated with vanadate (V1: 50%; V2: 45.8%; V3: 45.8%) than in the rats from the control group (C: 54.2%), but this difference was not statistically significant, $p > 0.05$. The diabetes onset age was significantly higher for groups V1 (84.82 ± 16.8 days), V2 (102.8 ± 33.5 days), V3 (105.5 ± 16.1 days) as against the control group C (73.9 ± 10.5 days), $p < 0.05$ (V2, V3 vs C), and depended on the vanadate concentration, $p = 0.006$ (V3 vs V1). The BG levels in groups V decreased slowly in week 1 (V1 from 95.7 ± 2.5 mg/dl to 63 ± 11 mg/dl, $p = 0.007$; V2 from 100.3 ± 2.5 mg/dl to 73 ± 6.5 mg/dl, $p = 0.0025$; V3 from 98 ± 2.8 mg/dl to 73 ± 5.6 mg/dl, $p = 0.03$) and increased slightly during in week 2. Before the diabetes onset (2 weeks), the rats in group C lost in BW from 239.5 ± 55.8 g to 208.5 ± 34.6 g while the rats in groups V presented a slight increase in BW from 309.4 ± 23.6 g to 344 ± 16.8 g, $p < 0.05$ (C vs V1, V2, V3). In diabetic rats, the BG at the onset was higher in group C (383.2 ± 48.2 mg/dl) than groups V (V1: 276.8 ± 103.4 mg/dl; V2: 286.2 ± 107.1 mg/dl), $p < 0.05$ (V1, V2 vs C). **Conclusions:** 1) Sodium vanadate does not prevent the onset of diabetes mellitus in BB-DP rats, but it can delay its development. 2) Vanadate can determine the occurrence of a milder diabetes mellitus. 3) The effects depend on the sodium vanadate concentration and 0.2 mg/ml is preferable.

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TRI-IODO-THYRONINE (T3) REDUCES THE INCIDENCE OF DIABETES AND INCREASES THE RELATIVE BETA-CELL MASS IN RATS.

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Background and Aim: It has been described that thyroid hormones are able to modulate the immune system, influence insulin secretion, and cause decreased glucose tolerance. The aim of this study was to investigate the influence of thyroid hormones on the incidence of diabetes in BB rats and on the beta-cell mass in Wistar rats.

Materials and Methods: During adolescence T3 was given in the drinking water to 36 BB rats from the age of 3 to 9 weeks and 43 BB rats at the age of 5 to 9 weeks (0.3 mg/L in the first 2 weeks and thereafter 0.2 mg/L). Control BB rats (n=32) received pure drinking water. The rats were sacrificed at the age of 120 days.

Since T3 was given during an important growth period, we also investigated whether this treatment changed the beta-cell mass. For this purpose, the treatment with T3 was given to 9 male Wistar rats at the age of 5 to 9 weeks. 11 male Wistar rats served as control group. After sacrifice at the age of 9 weeks the beta-cell mass was evaluated in accordance with unbiased stereological principles.

Results: T3 given to BB rats from the age of 3 to 9 weeks decreased the incidence of diabetes to 30.6% compared to 56.3% in the control group (p=0.03). T3 given to BB rats from 5 to 9 weeks of age also decreased the incidence of diabetes to 23.2% compared to 56.3% in the control group (p=0.007).

The beta-cell mass to body weight ratio in Wistar rats increased in the T3 treated rats (Mean±SEM) $2.2 \times 10^{-3} \pm 0.1 \times 10^{-3}$ compared to $1.8 \times 10^{-3} \pm 0.2 \times 10^{-3}$ in the control group (p<0.03).

Conclusion: T3 treatment of BB rats during the adolescence decreases the incidence of diabetes and increases the beta-cell mass per gram body weight. The preventive effect of T3 could be due either to modulation of the immune system, to decreased insulin secretion, or to the induction of a relatively higher beta-cell mass.

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ADMINISTRATION OF VITAMIN D3 DURING PREGNANCY AND EARLY LIFE DID NOT REDUCE DIABETES INCIDENCE IN NOD/BA MICE.

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Background and Aims: Recent literature describes a correlation between autoimmune disease and metabolism of vitamin D3. Some studies have suggested a lowering of diabetes incidence in at risk groups if vitamin D is given during pregnancy or in the early stages of life. This experiment used non-obese diabetic mice (NOD) to determine if diabetes incidence could be lowered in this model by vitamin D3 administration in utero & early stages of life.

Materials and Methods: Twelve breeding pairs of NOD/BA mice were treated with olive oil containing vitamin D3 (1000 IU/kg body weight) during pregnancy and for fifteen days following parturition to cover the period of maternal feeding. The pups were then given olive oil with vitamin D3 at the same dose until 10 weeks of age. After a 'wash-out' period of 15 days the same breeding pairs were allowed to produce a second litter but this time the parents & pups were dosed with a control liquid in order to produce a control group. Female mice were followed (29 treated and 33 control) and from 10 weeks of age onward were tested weekly for diabetes by testing for urinary glucose. Body-weights and food & water intake, were also taken weekly until the conclusion of the study at 30 weeks of age. Mice developing diabetes were removed from the study. At culling blood glucose was measured to confirm diabetic/non-diabetic status and internal organs removed and frozen for later analysis.

Results: There were 12/29 diabetic mice in the group treated with olive oil/vitamin D3 and 15/33 diabetics in the control group (p=0.899, log rank, not significant). There was also no delay in diabetes onset between the two groups. There was no difference between the two groups for any of the other measured parameters.

Conclusions: Vitamin D3 administered in utero and in the early stages of life did not alter the incidence of diabetes in NOD/BA mice.

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DIABETES-PROMOTING DIETARY PROTEINS CHANGE PROTEIN EXPRESSION IN RAT INTESTINAL EPITHELIAL CELLS

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Background and Aims: Oral exposure to soy and wheat gluten (WG) proteins is associated with increased frequency of type 1 diabetes in both animal models of spontaneous autoimmune diabetes, BioBreeding rats and non-obese diabetic mice.

Because ingested peptides cross the intestinal epithelium before reaching the gut associated lymphoid tissue, we investigated protein expression of rat intestinal epithelial cells (IEC-6) cultured with proteins or peptides of increasing diabetogenic potential: chymotrypsin digested WG > soy protein isolate > hydrolyzed casein (HC).

Materials and Methods: IEC-6 cells were incubated with 50 micrograms of protein and the cell lysates were analysed using 2-dimensional electrophoresis. Spot patterns were compared to distinguish the presence or absence of proteins with respect to untreated cells. Proteins of interest were separated by capillary liquid chromatography, sequenced using mass spectrometry and identified by comparison with published protein sequences. **Results:** There was a major reduction in protein expression after stimulation with diabetes-promoting proteins (WG and soy), similar to the response seen in the small intestine of celiac patients challenged with WG. Heat shock protein 40 and certain enzymes were absent or downregulated in cells cultured with WG or soy. However, Annexin II was expressed in cells cultured with WG and soy but not HC or untreated cells. Protein expression was enhanced in the presence of HC. By contrast with WG and soy treatments, cells exposed to HC expressed antioxidant enzymes such as thiol-specific antioxidant protein 2 and thioredoxin peroxidase, as well as Annexin V and galactokinase.

Conclusions: IEC-6 cells show a gradation in protein expression that is related to the diabetogenic potential of the protein source. Our findings suggest that exposure to HC, a diabetes retardant diet, induced an antioxidant response. Conversely, the response induced by diabetes-promoting WG and soy proteins is suggestive of gut damage. The pathogenesis of diet-induced type 1 diabetes may be related in part to a differential response of the gut epithelium to diabetes-promoting proteins. Proteomic analysis of IEC-6 cells is a useful tool to evaluate the interaction between these dietary agents and the gut epithelium.

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Autoimmunity I

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PREDICTIVE VALUE OF AUTOANTIBODIES TO IA-2 FOR INSULIN REQUIREMENT IN JAPANESE TYPE 1 DIABETES

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Background and Aims: Type 1 diabetes is evidently a heterogeneous disorder, and is often of slow onset known as latent autoimmune diabetes in adults (LADA). Autoantibodies to glutamic acid decarboxylase (GADA) are detected frequently in LADA and predict insulin dependency among diabetes. The aim of the study was to clarify the presence of newly developed antibodies to the protein tyrosine phosphatase IA-2 (IA-2A) and compare its clinical usefulness with GADA in Japanese type 1 diabetes. **Materials and Methods:** Sera from 61 Japanese patients with type 1 diabetes (45 acute onset type 1 diabetes, 16 LADA patients) attending to our hospitals (25M/36F; mean age 40.6 ± 13.3 yrs; duration 12.6 ± 9.8 yrs) were studied. Diagnosis of type 1 diabetes was determined clinically and confirmed by basal serum C-peptide (<0.5 ng/ml) or urine C-peptide excretion rate (<20 µg/day). Autoantibodies were measured by radioimmunoassay kit using recombinant human GAD65 (Cosmic, Tokyo, Japan) and ICA512 (RSR Ltd, Cardiff, UK). **Results:** Overall, GADA and IA-2A were detected in 34 (56%) and 24 (39%) of type 1 diabetes respectively. Forty-one (67%) type 1 diabetic patients had any of the antibodies, therefore, the frequency of either GADA or IA-2A in these subjects were higher than that of GADA or IA-2A alone ($p < 0.001$). Seventeen (27.9%) were positive for both autoantibodies. Of 16 LADA patients, GADA and IA-2A were detected in 12 (75%) and 8 (50%) respectively, whereas no statistical differences were found. The titer of IA-2A in GADA-positive patients were higher than in GADA-negative patients (1.57 ± 2.46 vs 0.49 ± 1.25 U/ml, $p < 0.05$), and that of GADA in IA-2A positive patients were higher than in IA-2A negative patients (66.3 ± 75.0 vs 17.7 ± 37.0 U/ml, $p < 0.02$). The higher were titers of IA-2A, the higher dose of insulin were required. The patients with IA-2A more than 4.0 U/ml in titer, corresponding to 20% of type 1 diabetic patients, required insulin doses over 20 units per day for treatment. **Conclusions:** IA-2A had more predictive value for insulin dependency in type 1 diabetes than GADA. IA-2A, alone or in combination with GADA may provide a useful diagnostic value for diagnosis and prognosis of patients with type 1 diabetes.

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AUTOIMMUNE INSULITIS IN FIRST-DEGREE RELATIVES OF DIABETIC PATIENTS IN THE CZECH REPUBLIC

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Background and Aims: Diagnosis of autoimmune insulinitis by genetic risk analysis, autoantibodies evaluation and functional test of stimulated insulin secretion performance in first-degree relatives of diabetic patients.

Materials and Methods: 208 Czech children and adolescents (101 boys and 107 girls, 186 siblings, 22 offspring of diabetic parents, aged 1-22 years, mean age 11.5 ± 5.4 years) were enrolled in the study. Complete DQB1, DQA1 typing and DRB1*04 subtyping were performed by the PCR in 202 subjects. Sera of all children were investigated for anti-GAD65, anti-IA2 and insulin autoantibodies (IAA) using RIA methods. The cut-off normal levels were determined as the 99th percentile of 105 non-diabetic children. IVGTT (ICARUS modification) was performed in children with significant titre of one or more autoantibodies. Total level of stimulated insulin secretion < 48 mU/ml was assessed as defect of FPIR.

Results: Risk genotype DQA1*05-DQB1*0201/DQA1*03-DQB1*0302 (OR=100, CI 95% 13-730 for Czech children) was found in 24 of 202 first-degree relatives (12%). 22 children (11%) carried strong protective allele DQB1*0602 (OR=0.03, CI 95% 0.01-0.12). Autoantibodies positivity was recognised in 9 of 208 children (2.9%) and IVGTT was performed. Positivity of anti-GAD65, anti-IA2 or IAA was identified in 5 of 24 children with the highest risk genotype (21%) and in 4 children of 113 with lower risk or neutral genotypes (3.5%). Borderline positivity of one autoantibody was found in 1 boy with the highest risk genotype and in 2 children with lower risk genotypes. Only temporary anti-GAD65 positivity was found in girl with protective genotype. Type 1 diabetes mellitus was diagnosed in boy during IVGTT and disease manifested 6 months after IVGTT in girl with defect of FPIR.

Conclusions: Standardised methods for secondary prevention of Type 1 diabetes were introduced in first-degree relatives of diabetic patients. These methods are used for Czech registry of diabetic children.

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ALSO INDIVIDUALS OF A NORMAL POPULATION WITH MULTIPLE OR HIGH TITER SINGLE AUTOANTIBODIES TO BETA-CELL ANTIGENS REVEAL AN INCREASED GENETIC TYPE 1 DIABETES SUSCEPTIBILITY

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Background and Aim: Type 1 diabetes is caused by specific destruction of beta-cells, accompanied by autoantibodies (AABs) to beta-cell antigens. The aim is to improve the strategy to recognise individuals of a general population at risk progressing to diabetes. **Materials and Methods:** 12,558 schoolchildren were initially tested for AABs to GAD (GADA), protein tyrosine phosphatase (IA-2A) and insulin (IAA) by radioassays, and AABs binding on pancreatic sections (ICA) by immunofluorescence. 203 children, representing 6,155 (3,086/3,069m) of a healthy child population, repeatedly AAB+ at levels $\geq 99^{\text{th}}$ centile, were HLA-genotyped. **Results:** 203/6115 (3.3%) were tested AAB+. 1.5%, 0.9%, 0.8%, 1.3% had GADA, IA-2A, IAA, ICA, respectively. The AAB+ children revealed a significantly increased ($P < 0.05$) frequency of the diabetes-associated DQB1 alleles *0302 and/or *02 and a decreased frequency of the protective allele *0602. Highest GADA and IA-2A level were found in children without the protective allele. 36 (0.6%) had multiple AABs. 97% (35/36) could be identified by GADA/IA-2A testing. None of them (0/36) had the protective allele *0602 and the frequency of the risk alleles *0302 and/or *02 increases as the AAB numbers from 51%, 72%, 92%, and 100% with 1, 2, 3, and 4 AABs, respectively. 9/36 (25%) with multiple AABs progressed already to type 1 diabetes. 89% (8/9) have the *0302 and/or *02 risk alleles in absence of the protective allele *0602 but only 11% (1/9) of the recent onset cases and 8.3% (3/36) of the total multiple AAB+ subjects had the highest risk genotype DR3/4(DQ2/8). **Conclusions:** The most of AAB+ individuals at risk could also be recognised by GADA/IA-2A testing of a normal population. HLA genotyping allows a further risk differentiation. Subjects with multiple AABs reveal the highest risk progressing to type 1 diabetes, also supported by significantly enhanced genetic diabetes susceptibility.

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AUTO-ANTIBODIES PROFILE AND BREAST-FEEDING DURATION IN TYPE 1 DIABETIC PATIENTS FROM CHILE

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Introduction: Type 1 diabetes, as an autoimmune disease presents several islet cell-specific autoantibodies such as islet cell antibody (ICA), anti-insulin (IAA), anti-glutamic acid decarboxylase (GAD) and anti-tyrosine phosphatase (IA-2). Between environmental factors associated to etiology of type 1 diabetes has been focused viruses, breast feeding duration and the early exposure to cow's milk proteins. **Aim, Patients and Methods:** In order to study the frequency of the anti-GAD, anti-IA-2 e ICA antibodies in Chilean type 1 diabetic patients and determine the possible modulator effect of the breast feeding, we studied 134 type 1 diabetic patients at diagnosis debut who were compared among them according to the breast feeding duration period. IA-2 and GAD were determined by RIA and ICA by means of indirect immunofluorescence. Statistical analysis by means of Wilcoxon and Student test. **Results:** The auto-antibodies frequency between patients with exclusive breast feeding ≤ 3 months and > 3 months was: ICA+ (78.8% vs 90.6%, NS), GAD+ (75.0% vs 54.6%, $p=0.024$) and IA-2+ (73.0% vs 43.8%, $p=0.001$). The simultaneous positive titles for 3 antibodies was 53.9% and 21.8% between children with breast feeding duration ≤ 3 months and > 3 months, respectively ($p=0.001$). The positive titles of IA-2 and GAD was 39.5% between children with breast feeding duration < 1 months (19/48). **Conclusion:** Both IA-2 and GAD exhibit a low positive frequency in type 1 diabetic patients with breast feeding duration higher than 3 months. The breast feeding duration at least by one month mean to be crucial, suggesting a possible attenuate role of exclusive breast feeding on the pancreatic autoaggression events in type 1 diabetes.

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TRANSGLUTAMINASE AUTOANTIBODIES ARE THE BEST MARKERS FOR CELIAC DISEASE SCREENING IN SPANISH TYPE 1 DIABETIC CHILDREN AND ADULTS

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Background and Aims: Prevalence of celiac disease (CD) in type 1 diabetes (DM-1) has been reported to be higher (1.7-5.6%) than in general population (0.01-0.3%). The aim of this study is to determine the prevalence of CD in children and adults with DM-1 in Alicante (Spain) and the value of serological markers for screening of CD in this population. **Materials and Methods:** 247 patients with DM-1 (mean age 26.5 ± 14.1 , range 3-74 years), from a total of 590 patients who were invited to participate in the study, accepted to be tested prospectively for gliadin IgA/IgG (AGA-IgA/IgG) antibodies and endomysial IgA antibodies (EMA) once a year during 3 years. These antibodies (ab) were also measured in frozen sera of 60 control subjects. Diabetic patients who were positive during follow-up for more than one of these antibodies were asked to undergo intestinal biopsy. We measured once recombinant human transglutaminase IgA ab (TgA), glutamic acid decarboxylase ab (GAD) and protein tyrosine phosphatase-2 ab (IA-2) and total IgA in frozen sera of all patients. **Results:** One DM-1 patient had known celiac disease before the study and was excluded. Of the 247 patients tested, 16 (6.5%) were IgA deficient and 64 (26%) were found to be positive for any antibody associated with celiac disease: 54 (17.6%) for AGA-IgA, 8 (5.1%) for AGA-IgG, 8 (3.4%) for EMA and 2 (1.2%) for TgA. Positive seroconversion of antibodies occurred in 17 patients and negative seroconversion in another 17 patients. Of 31 patients biopsied, 1 asymptomatic patient had total villus atrophy (silect CD) and 4 asymptomatic patients had an increase of intraepithelial lymphocytes (potential CD). The sensitivity and specificity of serological markers for villus atrophy in biopsied patients were: 100% and 32% for AGA-IgA, 100% and 73.9% for EMA, 100% and 96% for TgA and 100% and 3.8% for combined antibodies respectively. There was no association between the presence of antibodies related to CD and GAD or IA-2 antibodies in these patients. **Conclusions:** Prevalence of CD (including potential CD) in DM-1 patients in Alicante was 2.4%. IgA-Transglutaminase antibodies were the most sensitive and specific antibodies for CD screening in DM-1 patients.

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IMMUNOSCREENING A WHEAT CDNA LIBRARY FOR CANDIDATE PEPTIDES INVOLVED IN THE PATHOGENESIS OF TYPE 1 DIABETES

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Background and Aims: The development of type 1 diabetes requires genetic susceptibility and is influenced by environmental factors, such as viral infections and dietary components. Diets in which wheat gluten (WG) is the sole source of amino acids are associated with high diabetes frequency in the diabetes prone BioBreeding (BBdp) rat and non-obese diabetic (NOD) mouse, both models of spontaneous autoimmune diabetes. The identity of the peptides or proteins responsible for the diabetogenicity of WG remains unclear. It may be that, as in gluten-sensitive enteropathy, certain wheat peptides are not only immunogenic, but also diabetogenic. The objective of this study was to identify immunogenic wheat peptides that may be involved in the pathogenesis of type 1 diabetes in the BBdp rat. **Materials and Methods:** Total RNA was extracted from wheat kernels and a wheat cDNA expression library consisting of one million recombinants was constructed. To identify immunogenic wheat peptides, the library was screened with pooled serum from 7 diabetic WG-fed BB rats ranging in age from 73-121 days. Diabetes was diagnosed in animals with fasting blood glucose level > 11.1 mM. A secondary anti-rat IgG, Fc (gamma) fragment specific antibody conjugated to alkaline phosphatase was used to identify positive clones. Positive clones were screened repeatedly until clonality was achieved. The cDNA inserts from isolated clones were subjected to automated sequencing. Blastn and Blastx searches of Genbank and TIGR Wheat Gene Index databases were performed to identify homologous sequences. **Results:** Primary screening of the library resulted in 48 clones. Secondary and tertiary screening resulted in the isolation of 24 clones. Eight clones produced immunogenic peptides that shared 87-92% DNA sequence identity with the wheat storage protein (globulin 1) gene across 386 nucleotides. Three clones shared 100% DNA sequence identity with a putative Arabidopsis thaliana protein and the human IL-1 receptor antagonist gene across 21 nucleotides. **Conclusions:** These data show that antibodies from diabetic animals identified wheat peptides that share sequence similarity to (i) plant proteins and (ii) a cytokine receptor antagonist for IL-1. Considering that IL-1 is a mediator of beta cell destruction, this novel approach may identify molecular structures involved in the development of food antigen related type 1 diabetes.

(Supported by JDF/CHIR/ORDCF/Health Canada)

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Early growth and infant feeding and risk of Type 1 diabetes: a multicentre case-control study.

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Background and Aims: Recent studies describe not only increased height in early life as a risk factor for childhood type 1 diabetes but also increased ponderosity. Our aim was to determine if various measures of growth (height, weight and body mass index) in early life are associated with childhood type 1 diabetes in different European populations and to elucidate any role of infant feeding practices in the association. **Materials and Methods:** Five European centres with population-based registers of type 1 diabetes diagnosed under 15 years of age participated. Controls were randomly chosen from population registers, schools or policlinics. Routine growth data up to the date of diagnosis were extracted from records and information about breast feeding and introduction of cows milk formula and solid foods was obtained from parents by questionnaire or interview. Case control differences in mean standard deviation score (SDS) were obtained for each centre and pooled. Odds ratios were pooled over centres using the Mantel-Haenszel method. Logistic regression was used to adjust for confounders. **Results:** Growth data were available for 499 cases and 1337 controls. Both height and weight SDS were significantly increased among cases from one month after birth with the maximum differences in SDS of 0.32 (95% CI 0.14, 0.50) and 0.41 (0.26, 0.55), respectively, occurring between one and two years of age. Significant excesses in body mass index SDS were observed from 6 months of age, with the largest difference of 0.27 (0.10, 0.44) again evident between one and two years. Both height and body mass index SDS between one and two years of age remained significant predictors of risk when jointly fitted in a logistic regression with confounding variables. Breast-feeding was associated with a significant reduction in risk; odds ratio 0.75 (95%CI 0.58, 0.96). Contrary to previous reports, early introduction (before three months) of cows' milk or formula or of solid foods was not associated with significant elevation in risk. **Conclusions:** Increased early growth measured either by height or body mass index, is associated with an increased risk of childhood diabetes in a variety of European populations. Any role played in this association by infant feeding practices remains unclear, but we speculate that relative overnutrition of children causing an increased growth rate may overload the beta cells and thereby increase the risk of type 1 diabetes in susceptible children.

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AUTOANTIBODIES TO TISSUE TRANSGLUTAMINASE (TTG-Ab) IN PROTEIN DEFICIENT DIABETES (PDDM) AND FIBROCALCULOUS PANCREATIC DIABETES (FCPD) PATIENTS FROM EASTERN INDIA.

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Background and Aims: Antibodies to tyrosine pyrophosphatase (IA2-Ab) and glutamate decarboxylase65 (GAD65-Ab) are major markers for IDDM in caucasians. Tissue Transglutaminase antibodies (TTG-Ab) are specific for celiac disease. Celiac disease is characterized by small intestinal damage with loss of absorptive villi and hyperplasia of the crypts, typically leading to malabsorption. Celiac disease is precipitated by ingestion of the protein gliadin, a component of wheat gluten, and usually resolves on its withdrawal. 10% to 20% of the Celiac disease patients are also have IDDM and both disease are associated with HLA-DR3-DQ2. The aim of the study was to estimate the prevalence of TTG-Ab in MRDM (n=128), IDDM (n=74) and NIDDM (n=216) and 122 healthy controls from Cuttack in Eastern India.

Materials and Methods: Protein deficient diabetes (PDDM) studied were 71 and Fibrocalculus pancreatic diabetes (FCPD) were 47. MRDM patients are typically young at onset with low body mass index, require insulin treatment for glycemic control, have insulin resistance and do not develop ketosis on withdrawal of insulin. FCPD, but not PDDM, patients have abdominal pain and calculi in the pancreas. In the revised classification, PDDM is referred to as Malnutrition modulated diabetes mellitus (MMDM). TTG-Ab was evaluated by RIA using in vitro translated recombinant human 35S-TTG.

Results: In controls, TTG-Ab was present in 3/122 (2%). In PDDM, TTG-Ab was present in 14/71 (20%)

(p<0.0001 vs controls) and in FCPD 8/46 (17%) were positive for TTG-Ab (p<0.005 vs controls). 11/74 (15%) IDDM (p<0.05 vs controls) and 23/216 (11%) NIDDM (p<0.05 vs controls) were also positive for TTG-Ab.

Conclusions: We conclude that MMDM, FCPD, IDDM and NIDDM from Cuttack in Eastern India, have significantly high proportion of TTG-Ab compared to healthy controls. The highest significance is seen with MMDM patients. It is important to note that subclinical Celiac disease exists with diabetes mellitus and must be considered in the diagnosis of MMDM.

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MECHANISMS OF BETA CELL DEATH DURING RESTRICTED AND UNRESTRICTED COXSACKIEB VIRUS INFECTION.

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Background and Aims: Enterovirus infections may trigger and accelerate beta-cell death leading to IDDM. Especially coxsackie B-strain viruses (CBV) are potentially diabetogenic. Productive CBV-infection induces pyknotic morphological changes and functional impairment in human beta cells. Our aims were to 1) generate a model more closely resembling the beta-cell infection in vivo by restricting the production of viral progeny and 2) investigate the mechanisms of virus-induced beta-cell death during productive and restricted infective conditions.

Materials and methods: Porcine fetal pancreatic cells (FPF) and MIN6 mouse insulinoma cells were infected with a prototype strain of CBV5. Mechanisms of cell death were studied by nuclear viability staining (5 µg/ml Hoechst 33342 and 1.5 µM ethidium homodimer-1) and electron microscopy. Guanidine hydrochloride (G-HCl) was used as a selective inhibitor of coxsackie-virus replication. The protective capacity of G-HCl was tested in 0.1, 0.5, 1 and 5 mM concentrations. Results: CBV5 infection significantly decreased the viability of both MIN6- and FPF-cells (MIN6 viability at 36h 78±6%; FPF viability at 7d 22±5%). Throughout the viral exposure 1 mM G-HCl was the lowest effective concentration to inhibit CBV5-induced cell death and preserved MIN6 viability at 96±1% (p<0,01) and FPF viability at 69±5% (p<0,0001). While decreasing necrotic cell death, 1 mM G-HCl significantly induced apoptotic activity in FPF-cells (CBV5 4,5±1%; CBV5 + 1 mM G-HCl 7,2±1%, p<0,05), and the ratio of apoptotic/necrotic cells increased significantly (CBV5 0,1±0,04; CBV5 + 1 mM G-HCl 0,46±0,1, p<0,0001).

Conclusions: Restriction of virus replication with G-HCl effectively decreases CBV5-induced cell death both in insulinoma cells and primary pancreatic beta cells. The lowest protective concentration within the selective anti-viral range is 1 mM. However, restriction of viral replication shifts the mechanism of cell death towards apoptosis.

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ENTEROVIRUS INFECTIONS AND TYPE 1 DIABETES- INTERNATIONAL COMPARISON

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Background and Aims: Enterovirus infections have associated with increased risk for Type 1 diabetes in retrospective case-control studies as well as in prospective studies. To study further this association we analysed if the frequency of enterovirus infections correlates with the incidence of Type 1 diabetes when compared between countries with either high or low incidence of diabetes.

Materials and Methods: Frequency of enterovirus infections in different populations was studied by analysing enterovirus antibodies in 10-14-year old children in Finland, Sweden, Russia, Estonia, Lithuania, Hungary and Germany. Serum samples (N=120,114,114,106,103,91,104 respectively) were analysed for IgG class antibodies against purified coxsackievirus B4, poliovirus Type 1 and synthetic enterovirus peptide using EIA. The frequency and levels of these antibodies reflect past exposure to enteroviruses and the frequency of enterovirus infection in a given population.

Results: The levels of enterovirus antibodies varied a lot between different countries. Median levels (EIU) of peptide antibodies were 42 (Estonia), 19 (Finland), 42 (Germany), 14 (Hungary), 31 (Lithuania), 30 (Sweden), 16 (Russia). The incidence of type 1 diabetes (/100 000) is 10, 37, 11, 6, 6, 25, 7 respectively. Poliovirus antibodies, which arise from polio vaccinations, varied also considerably from the median of 18 EIU in Finland to 42 EIU in Germany.

Conclusions: The levels of enterovirus antibodies varied considerably between different countries suggesting variation in the transmission of enterovirus infections. The populations with high incidence of Type 1 diabetes (like Finland) did not have exceptionally high levels of enterovirus antibodies suggesting that high incidence of type 1 diabetes is not necessarily associated with a high frequency of enterovirus infections in the population. The differences in poliovirus antibody levels may reflect differences in the vaccination programmes.

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Heterogeneity of Type 1 diabetes by age at onset: the Registry of the Province of Turin, Italy

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Background and Aims: Heterogeneity in Type 1 diabetes according to the age at onset of the disease has been suggested, which might reflect differences in determinants of the disease. Few registries have expanded the registration up to the adults. The registry of the Province of Turin is currently collecting incident cases up to age 29 yrs. In addition, since 1999, a pilot study has been started to collect incident cases of Type 1 diabetes and LADA up to age 54 yrs. Aims of this study were to assess clinical and immunologic heterogeneity of the disease by age at onset.

Materials and Methods: Patients were identified through the diabetes clinics of the area. A cohort of 282 patients was included in this study: 171 were aged 0-29 at the onset of the disease and 111 were aged 30-54 yrs (n. 62 type 1 and n. 49 LADA). By two months from the diagnosis, fasting and 6 min after 1 mg glucagon e.v. plasma C-peptide, ICA and GADA were measured in a centralized laboratory

Results: BMI (mean, SD) was higher in adults patients with LADA than in adults with Type 1 diabetes: 24.8 (4.9) vs 22.3 (2.9). A decreasing linear trend of fasting plasma glucose by age at onset was found (p<0.001). Glycosuria was found in only 52.1% of patients with LADA vs. 72% of patients aged 30-54 and 96% of those <30 yrs. Fasting C-peptide was 0.14 (0.10) in age 0-9, 0.26 (0.17) in age 10-19, 0.36 (0.26) in age 20-29, 0.40 (0.31) in age 30-54 and 0.69 (0.32) in LADA (p<0.001). A linear trend of stimulated C-peptide with age was also found (p<0.001). In type 1 diabetes, prevalence of patients with both negativity to ICA and GADA was 11% in patients aged 30-54 yrs and only 4.3% in those aged 0-29 yrs. Lower frequency of patients with type 1 diabetes had ketonuria at the onset: 89% in age 0-29 and 40.7% in age 30-54 yrs (p<0.001).

Conclusions: this study confirms the heterogeneity of type 1 diabetes, with better preserved beta cell function in children than in adults and higher frequency of adults patients with negativity to both ICA and GADA.

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Autoantibodies to IA2ic and to Phogrin increase the prediction for insulin requirement in patients with Type 2 Diabetes

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Background and aims: ICA and GADA are detected in a subset of Type 2 diabetic (T2D) patients (pts) and their presence is correlated with the need for insulin requirement (IR) within 6 years from diagnosis. Little is known about the prevalence of autoantibodies (AA) against IA2 autoantigens and their role in the prediction of the IR in T2D pts. Thus, we carried out a retrospective study in these pts aimed to investigate the frequency of two different IA2 AA, the ones recognising the intracellular fraction of the IA2 (IA2icA) and the ones related to IA2b (IA2bA) or phogrin. Age at onset, clinical and metabolic characteristics, and HLA genotyping have been correlated with the presence of the two IA2 AA investigated. **Materials and Methods:** IA2icA and IA2bA were measured in 3,600 white Caucasian newly diagnosed T2D pts, aged between 25 and 65 years, recruited to the UKPDS. The need for IR within 6 years from diagnosis has been evaluated in 2,263 pts, who were not assigned to insulin. Both IA2icA and IA2bA were measured by radioimmunoassay with human recombinant autoantigens. DR3/DR4 genotyping was available in 1,342 pts. **Results:** IA2icA and IA2bA were detected in 93 (2.6%) and 58 (1.6%) pts, respectively. IA2icA alone were present in 42 (1.2%) pts, IA2bA alone in 7 (0.2%), whereas a combination of the two IA2 AA was detected in 51 (1.4%) pts. Due to the small number of pts positive for IA2bA alone, the analysis has been carried out only by considering the 93 IA2icA positive pts. IA2icA positive pts showed higher levels of FPG, HbA1c and insulin sensitivity, and lower values of age at diagnosis, BMI and Bcell function, compared to the pts without IA2icA (p<0.0001 for all variables). The proportion of pts with IA2icA decreased significantly with the age at diagnosis (16.2% in the age group 25-35 years and 1.2% in the age group 55-65 years; p<0.0001). The frequency of DR4 allele was significantly higher in IA2icA positive pts than in the negative ones (66% vs 36%, respectively; p<0.001). The sensitivity of IA2icA for IR was 17.5%, while the positive predictive value was 73% compared to 8.9% registered within IA2icA negative pts (OR 15.2; 95% CI 5.56-41.5). **Conclusions:** In T2D pts, the presence of IA2icA is highly predictive of the need for the future IR, despite being of low sensitivity. As in Type 1 diabetes, IA2icA are frequently associated with the DR4 haplotype and are more likely to be found in younger pts. The measurement of IA2bA does not provide any additional information.

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Clinical Characteristics of Latent Autoimmune Diabetes of the Adult in the United States

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Background and Aims: Latent Autoimmune Diabetes of the Adult (LADA) is a distinct subtype of diabetes mellitus that is frequently misclassified as type 2 diabetes mellitus (T2DM). Our aim is to describe and compare the clinical characteristics of patients with LADA, with those of T2DM, in the U.S.A.

Materials and Methods: 39 patients were identified as having LADA [1] Insidious onset of diabetes at age more or equal to 30, 2) Initial diagnosis of T2DM with no insulin treatment for the first 12 months, 3) Presence of anti-GAD antibodies]. A control group of 39 patients with T2DM and negative anti-GAD antibodies were matched for age, sex, race, and duration of diabetes. History and physical examinations were performed and blood was obtained for measurement of: anti-GAD ab., serum C-peptide, plasma glucose, lipid profile, uric acid and serum creatinine. The Urine albumin/creatinine ratio was measured. Results were compared using the two tailed unpaired t test. Results are reported as means \pm SEM. P values of more than 0.05 were considered non significant.

Results: Groups were well matched for age (60.1 ± 1.6 vs. 60.1 ± 1.6 yrs., $p = n.s.$), duration of diabetes (10.0 ± 1.9 vs. 10.6 ± 1.0 yrs, $p = n.s.$), race (LADA: 33 whites/6 blacks; T2DM: 33 whites/6 blacks) and gender (LADA: 24 women/15 men; T2DM: 24 women/15 men). The BMI was significantly lower in the LADA group (25.3 ± 0.7 kg/m² vs 32.5 ± 1.0 kg/m², $p < 0.001$). The serum C-peptide levels were much lower in the LADA group than in those patients with T2DM (1.0 ± 0.2 vs 5.1 ± 0.4 ng/ml, $p < 0.0001$). The lipid profiles in the LADA vs the T2DM group demonstrated very similar LDL levels (111.5 ± 5.6 vs 117.4 ± 5.0 mg/dl, $p = n.s.$), total Cholesterol (184.2 ± 4.6 vs 189.7 ± 5.4 mg/dl, $p = n.s.$), IDL (16.9 ± 2.1 vs 19.3 ± 1.3 mg/dl, $p = n.s.$) and Lp(a) (9.5 ± 1.5 vs 7.0 ± 0.7 mg/dl, $p = 0.09$). There were significant differences in: total HDL (57.3 ± 3.2 vs 37.6 ± 2.1 mg/dl, $p < 0.001$), HDL3 (37.3 ± 2.2 vs 28.5 ± 1.2 mg/dl, $p < 0.001$), HDL2 (15.8 ± 2.3 vs 7.7 ± 1.0 mg/dl, $p < 0.001$), VLDL (18.3 ± 2.2 vs 38.7 ± 3.1 mg/dl, $p < 0.001$), VLDL3 (7.6 ± 2.1 vs 13.6 ± 1.2 mg/dl, $p < 0.001$), Triglycerides (103 ± 9.9 vs 212.4 ± 18.3 mg/dl, $p < 0.001$) and LDL subfractions 1 - 4 (1.9 ± 0.2 vs 2.5 ± 0.1 , $p = 0.01$). While the serum creatinine was not different between both groups (1.0 ± 0.1 vs 1.0 ± 0.1 mg/dl, $p = n.s.$), the LADA group had a lower urine albumin/creatinine ratio (10.1 ± 5.3 vs 219 ± 111 , $p = 0.04$). Similarly the LADA group had a significantly lower serum Uric Acid (3.9 ± 0.3 vs 5.8 ± 0.5 mg/dl, $p = 0.001$).

Conclusions: LADA in the United States demonstrates significant differences in clinical presentation, including lower BMI, C-peptide, Uric Acid and a much different lipid profile, when compared to T2DM.

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CLINICAL CHARACTERISTICS, β -CELL FUNCTION, HLA TYPING AND HNF-1 α GENE MUTATIONS IN IMMUNOLOGICALLY NEGATIVE TYPE 1 DIABETES MELLITUS

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In our experience, around 20% of subjects with type 1 Diabetes Mellitus (DM1) (aged >15 yr.) are negative for pancreatic autoantibodies, this being one of the characteristics of the category DM1 B proposed by ADA. In light of the scarce information concerning this heterogeneous disorder, our study aimed to investigate clinical, metabolic and β -cell function characteristics, HLA typing and the presence of mutations in our area's most frequent MODY gene subtype (HNF-1 α) in immunologically negative DM1 subjects (Ab-). **Patients and methods:** 23 cases (25.5 ± 5.5 yr, 16 men) of Ab- patients (ICA, IA-2, GAD and IAA autoantibodies) and 23 age and sex matched Ab+ DM1 controls were included. Metabolic, clinical data and β -cell function (basal and glucagon stimulated c peptide) were evaluated at onset and after 12 m of follow-up. HLA-DRB1 typing (PCR method) from the genomic DNA was performed. Mutations in the HNF-1 α gene were analyzed by SSCP. **Results:** There were no differences between both groups in terms of BMI, insulin dose, clinical presentation and β -cell function at onset. After one year, there were no differences in terms of HbA_{1c}. At the same time, the AUC (area under curve) of stimulated c peptide in Ab- was significantly higher than in Ab+ (4.9 ± 4.0 vs. 3.14 ± 1.68 nmol/l, $p = 0.04$). Ab- and Ab+ displayed similar HLA-DRB1 typing (4/23 subjects in both groups showed DRB1*03*04; 19/23 subjects Ab- and 21/23 Ab+ displayed DRB1*03 and/or *04). We couldn't identify any mutation in the HNF-1 α gene in either group. **Summary:** Ab- and Ab+ DM1 subjects are similar in terms of clinical presentation, β -cell function at onset, metabolic control and HLA-DR genotype. Mutations in HNF-1 α gene would not explain insulin secretion defects in Ab-group. However, they display a better prognosis in terms of β -cell function than Ab+, at least during the early stages of the disease.

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Early correct classification in the Diabetes Syndrome-better long-term results.

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Background: Early correct diagnosis of diabetes mellitus means early adequate therapy. Misdiagnosis results in inadequate initiating treatment, which may cause either unnecessary inconvenience for the patient, or induce early late chronic complications as well. **Aims:** To differentiate between type 1 and type 2 diabetes based on different clinical and laboratory data. We selected 50 patients- 31 males, 19 females- with uncertain typology from our outpatient diabetic clinic. Age at diagnosis 34.2 ± 5.2 year, BMI: 24.2 ± 1.8 kg/m². In all of them oral diabetic drugs were initiated. **Methods:** to obtain more precise diagnosis were as follows: family history, how the diagnosis was set up, typical symptoms present or absent, fasting and postprandial blood sugar values and endogenous insulin levels, Haemoglobin-A1c, and autoimmune markers: islet-cell cytoplasmic antibodies, antibodies to glutamic-acid-decarboxylase and antibodies IA.2. **Results:** in spite of having typical diabetic signs, we found 4 classical type 1 patients, who were treated from 3- to 12 month with oral drugs. One became finally ketoacidotic, before switching her to insulin treatment. 14 patients were reclassified into the „slowly progressing type 1" (LADA) subform, however in only 8 patients were autoimmune markers positiv. In the remaining 6, rapidly diminishing endogenous insulin levels together with chronic hyperglycaemia proved the diagnosis. 2 patients became diabetic during pregnancy, but because of early exogenous insulin need, they also were reclassified as type 1 patients, with negative autoimmune markers. 2 young patients belong to the „maturity onset type diabetes in the youth" (MODY), 14 patients were classified as early onset type 2 patients continuously kept on oral drugs and finally in 10 patients exact classification remained uncertain. Frequency and severity of specific late complications in the LADA group presented large variation. **Conclusion:** even using all the clinical, biochemical and immunological parameters, in part of our diabetics final exact diagnosis may be uncertain for a long periode. In these cases insulin therapy must be initiated early in order to avoid or delay presence of late specific complications.

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LADA type of diabetes among Prague urban unselected diabetic patients

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Background and Aims: Last decade brought the evidence about the significance of type 1 diabetes manifested after 35 years of age was brought. To describe situation in Prague urban diabetic patients we decided to diagnose LADA type diabetes in out - patients of our department.

Materials and Methods: The total 306 patients were enrolled in the study. In all patients their BMI, fasting C - peptide level and level of antibodies for glutamate decarboxylase (GADA) were measured. The patients were divided to the groups according their BMI (less than 25, 25 - 29, over 30).

Results: 140 patients with BMI less than 25 were investigated. In 35 of them the fasting C - peptide level was below 200 pmol/l, 105 had fasting C - peptide over 200 pmol/l. From 35 patients with low C peptide were 18 GADA positive (over 50 ng/ml), 17 GADA negative, signs of autoimmune insulinitis was also 1:1. In 104 lean patients with fasting C peptide over 200 pmol/l 11 GADA positive and 94 GADA negative patients were found, relations between both group was 1:9. Difference in GADA positivity in low fasting C peptide and normal fasting C peptide patients was statistically significant (p less 0,01). 125 patients patients had BMI 25 - 29. In 20 of them was fasting C peptide less than 200 pmol/l, 8 had GADA over 50 ng/ml, 12 less than 50 ng/l, signs of autoimmunity was in 40 % of all low fasting C peptide group. In 105 patients with fasting C peptide over 200 pmol/l of this group had 12 positive GADA, other 93 were GADA negative. The ratio between both group was again 1 : 9. Difference GADA positivity in low fasting C peptide and normal fasting C peptide patients was statistically significant (p less 0,01). From 41 patients with BMI over 30 we found only 5 patients with fasting C peptide less than 200 pmol/l, of them 4 had GADA over 50 ng/ml. In other 36 patients fasting C peptide was over 200 pmol/l, only one of those patients had positive GADA.

Conclusions: We conclude, that from our group of 306 patients with diabetes diagnosed after 35 years of age we found in 60 of them (20,5%) fasting C peptide less than 200 pmol/l, half of them (i.e. 10% of all patients) had also markers of autoimmune insulinitis. Proportion of low fasting C peptide decreased with increasing BMI. Half of all patients with low C peptide (i.e. 30 patients) had presence of plasma increased GADA levels. LADA type diabetes also present important clinical problem. Supported from research Grant IGA Ministry of Health of Czech Republic No 5043/3

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C-Peptide in the Detection of Latent Autoimmune Diabetes of the Adult
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Background and Aims: Detection of latent autoimmune diabetes of the adult (LADA) at the time of diagnosing diabetes is important because prompt initiation of intensive insulin therapy may protect the remaining functioning beta cells from further immune destruction by decreasing their activity. This in turn might lead to easier glycemic control with less hypoglycemia and less complications. However, screening type 2 diabetic patients with anti-GAD antibodies is expensive. This study was performed to assess C-peptide levels could be used to screen for LADA.

Materials and Methods: Random serum C-peptide levels (normal range 0.8 to 4.0 ng/ml) were measured in 39 subjects with LADA (defining criteria: 1) Insidious onset of diabetes at age ≥ 30 , 2) Initial diagnosis of T2DM with no insulin treatment for the first 12 months, 3) Presence of anti-GAD antibodies). 39 subjects with anti-GAD negative type 2 diabetes Mellitus (T2DM), matched for age, race, sex and duration of diabetes, were used as controls. The statistical comparison was made utilizing the two tailed unpaired t test. Results are expressed as means \pm SEM.

Results: The mean C-peptide in the LADA group was 1.0 ± 0.2 ng/ml (range 0 to 4.3) vs. 5.1 ± 0.4 ng/ml (range 1.0 to 11.8) in the T2DM group. This difference was statistically significant ($p < 0.00001$). Only one subject (2.5%) in the LADA group had a C-peptide above the normal range (4.3 ng/ml, 7.5% above upper limit of normal). Meanwhile, all subjects with T2DM had a C-peptide within or above the normal range.

Conclusions: LADA can be ruled out in those patients presenting with adult onset diabetes by and elevated C-peptide. Testing for anti-GAD antibodies should be reserved for those patients found to have a normal or low C-peptide at the onset of diabetes.

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PREVALENCE AND CHARACTERISTICS OF LADA AMONG GREEK PATIENTS WITH THE INITIAL DIAGNOSIS OF TYPE II DIABETES

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Background and aims: Very little is known about the prevalence of LADA (Latent Autoimmune Diabetes in Adults) in Greek patients. Thus, the aim of the present study was to investigate the prevalence and characteristics of LADA (defined as GADA positive diabetes with age at onset greater than 35 years) among Greek patients with the initial diagnosis of type II diabetes. **Materials and methods:** In a total of 219 type II diabetic patients (99 men, 120 women) with a mean age of 67.8 ± 8.4 years and a mean diabetes duration of 14.1 ± 7.9 years GADA (Glutamic Acid Decarboxylase Antibodies) as well as ICA (Islet Cell Antibodies) were measured. Results were expressed as negative, positive and strong positive, according to autoantibody titres. **Results:** Prevalence of LADA was 26% in our patients. GADA positive patients did not differ significantly from GADA negative patients in age, sex, BMI, diabetes duration and prevalence of retinopathy, microalbuminuria, coronary artery disease and hypertension. However, GADA positive patients had a significantly lower prevalence of dyslipidemia ($p = 0.0002$) and a significantly higher prevalence of insulin dependency ($p = 0.0041$) than GADA negative patients. Degree of GADA positivity showed even stronger positive correlation with insulin dependency ($p = 0.0001$). Among GADA positive patients an ICA prevalence of 21% was demonstrated. GADA and ICA were positively correlated with regards to simultaneous presence in the same patient and to degree of positivity ($p = 0.01$). **Conclusions:** In a considerable percentage (26%) of Greek patients initially diagnosed as having type II diabetes presence of LADA is demonstrated by means of GADA measurement. Patients with LADA show a lower prevalence of dyslipidemia and a higher prevalence of insulin dependency than true (GADA negative) type II diabetic patients.

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Phenotypic and laboratory characteristics of 'gray zone' diabetes

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Background and Aims: The distinction between autoimmune and non-autoimmune diabetes becomes increasingly blurred during early adult ages and is termed 'gray zone diabetes'. To differentiate between these two types of diabetes, we evaluated a sample of 162 early-adult onset diabetes cases according to their phenotypic characteristics and laboratory parameters.

Materials and Methods: The study population consisted of 162 patients with diabetes started between 20-40 years of age. We divided the study population retrospectively according to early insulin requirement (within 1st year of diabetes).

Results: The first group included 50 cases mean age was 32.7 ± 7.0 yrs, age-at onset 29.4 ± 5.6 yrs, male 48 %, BMI 25.5 ± 4.7 kg/sqm, family history for diabetes 71 %, presentation with acute hyperglycaemia 30 %. Remaining 112 patients did not need early insulin and made up the second group (age 38.8 ± 6.2 yrs, age-at onset 33.5 ± 4.6 yrs, male 46 %, BMI 27.9 ± 4.3 kg/sqm, family history for diabetes 82 %, presentation with acute hyperglycaemia 11 %) Difference for age ($p < 0.001$), age-at onset ($p < 0.001$), BMI ($p = 0.002$) and type of presentation ($p = 0.002$) were statistically significant between two groups. Early insulin requirement correlated with preliminary diagnosis ($r = 0.24$, $p = 0.002$), final diagnosis ($r = 0.24$, $p = 0.002$), age-at onset ($r = 0.38$, $p < 0.001$), type of presentation ($r = 0.23$, $p = 0.003$), and HbA1C value ($r = -0.19$, $p = 0.044$). Where as we did not find significant correlation with early insulin requirement and gender, ICA, AntiGAD, low basal C peptide level ($p < 0.6$ ng/ml), family history and BMI.

Conclusions: These findings indicate that phenotypic characteristics are more predictive than laboratory results two differentiate 'gray zone' diabetes such as late type 1 or early type 2 diabetes.

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Epidemiology – Type 2 Diabetes: Prevalence Studies

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DIABETES IN THE ETHIOPIAN JEWISH COMMUNITY OF HADERA: PREVALENCE, ATHEROSCLEROTIC RISK FACTORS.

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Background and Aims: Studies on the prevalence of diabetes in the Israeli Ethiopian immigrant population indicate rapid growth as a function of years after immigration. These studies, however, were performed on people in transition; i.e. absorption camps, agricultural schools, etc., in which the particular conditions of life may have affected the development of diabetes. **Materials and Methods:** In this study, which is part of the Tene Briut# project, we examined the prevalence of diabetes and atherosclerotic risk factors in a representative sample of 15 years or older individuals from the Jewish Ethiopian community residing in Hadera. Diagnosis of diabetes was made based on fasting blood glucose level and post 75-gram oral glucose tolerance test (OGTT), in accordance with the new WHO criteria. **Results:** Up to now, we studied and analyzed data from 134 individuals randomly selected out of the 330 subjects of the total sample. Mean age was 53.8±11. Different age groups were equally represented. Twenty-four subjects were found to have diabetes (17.9%), 19 women and 5 men. The mean age was 60±17. Eight out of them were newly diagnosed (33%). Impaired glucose tolerance [IGT] was found in 17, seven women and 10 men, mean age 55±13. Ten had impaired fasting glucose [IFG], 6 women and 4 men, mean age 66±10. Twenty-eight subjects out of 134 were found to have hypertension (20.9%) 18.3% were newly diagnosed. But, in the diabetic group 46% also had hypertension. Hyperlipidemia was found in 16.2% with HDL-cholesterol below 35mg/dl, 16.6% had LDL above 160mg/dl and 9.3% had Triglyceride above 200 mg/dl. The BMI of the entire sample was 24.2 ± 2 while it was only 19 on arrival to Israel. The diabetic patients' BMI was 27.5±5, 27.3±4 in the IFG, and 24.3±4 in the IGT group. Fast and post-OGTT serum insulin concentration were 12.1 to 51.9 for normal OGTT subjects, 18.7 to 55.7 for IFG, 17.2 to 141.6 in the IGT and 12.8 to 58.5 in the diabetic patients respectively. **Conclusions:** This is the first report from the study. Although it is not complete, we can conclude that this population developed in a short period i.e., ten years since their arrival to Israel; obesity, high prevalence of diabetes and other atherosclerotic risk factors. Israeli Ethiopian immigrants are at high risk for cardiovascular diseases. This calls for in depth and culturally appropriate intervention programs in parallel with continuing study of the nature of the disease.

Project for education, prevention, detection and treatment of diabetes in the Ethiopian immigrants to Israel.

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Prevalence of gestational diabetes in a rural community of Bangladesh

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Background and Aims - About 7% of all pregnancies are complicated by gestational diabetes mellitus (GDM). Fasting blood glucose (FBG) ≥5.8 mmol/l in pregnancy is associated with increased risk of intrauterine death during the last 4-8 weeks of pregnancy. There are few data on the prevalence of GDM in Bangladesh and in other South East Asian communities despite increased perinatal mortality. The study addresses the prevalence of GDM in a rural population, which comprises more than 80% of the total population of Bangladesh.

Materials and Methods - Ten villages in a rural community with a population of 14,382 (M / F = 7476 / 6906) were selected. All married women of age group 15 - 40y were interviewed. Only pregnant mothers with gestational age over 20wk were investigated at home for obstetrical history, clinical examination, blood pressure and FBG. Capillary FBG was assessed by Hemocue cuvette.

Results - Of the total 2205 interviewees 172 (7.8%) were pregnant. Of them, 85.5% had gestational age over 20wk and 19.5% were multipara. About 20% experienced one or more unexpected abortion and 10% had one or more stillbirth. Their mean (SD) age was 25.8y (5.7) and BMI was 19.7 (2.2). Taking the fasting blood glucose 5.8mmol/l as a cut point, the prevalence of GDM was 4.8%. If the cut-point is taken at 5.3 mmol/l the prevalence rate reaches 7.5%. The GDM prevalence was found almost two-folds in those with history of two or more abortion compared with those who had one or no abortion (14.3 vs. 7.1%). Likewise, multipara also had two-fold increased prevalence of GDM (25 vs 13%).

Conclusion - The prevalence of GDM in rural Bangladesh is comparable to any other population with higher prevalence of GDM. Excess morbidity and mortality among mothers and newborns in a rural community may, in part, be due to hitherto unexplored increased prevalence of GDM.

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The prevalence of type 2 diabetes mellitus in rural and urban population over 35 years in Lublin Region (Eastern Poland)

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Background: The prevalence of diabetes mellitus type 2 has increased dramatically in the last decades. Asymptomatic course of disease causes that it is still detected too late. Data relating to the number of undetected cases of diabetes are underestimated.

The aim: The evaluation of the prevalence of type 2 diabetes mellitus, obesity, hypertension, and lipid disturbances in a representative group of urban and rural population in the Lublin region (Eastern Poland).

Material and methods: The study was performed in the period 1998-2000. A two-layer draw was applied: two groups of 3.000 people were drawn, from the population of Lublin town and from the rural areas each comprising 100.000 inhabitants. In all subjects we performed physical examination, body weight, height, and blood pressure measurements, oral glucose tolerance test (OGTT) after a 75g glucose load, and laboratory assessments: blood lipids and serum insulin concentration during OGTT. Diabetes mellitus was identified according to the WHO criteria from 1985. Obesity and hypertension were diagnosed according to the WHO criteria.

Results: The prevalence of diabetes was assessed in 17,6% of rural population and in 14,1% of rural population, respectively. More than 75% of diabetics in the rural areas and 56% in the town are the newly diagnosed patients. We found obesity in 30,8% of rural and in 30,1% of urban population. In female groups the prevalence of obesity was found in 39% of rural and 34 % of urban population. Obesity was associated with dyslipidaemia in 67,2%, with arterial hypertension in 84,9% and with impaired glucose intolerance or diabetes mellitus in 56,2%; we found a cluster of these 3 disturbances in 35,9% of all obese subjects. Isolated obesity was detected only in 6,5% of the obese in rural and in 10% of the obese in urban population.

Conclusion: Our results are very alarming. The data show a very high risk of atherosclerosis and diabetes and its later complications and are more dangerous than data from other studied groups. Our results point to the urgent need for an introduction of a national program for early diagnosis and prevention of diseases and exposure factors in high-risk groups.

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The Epidemiologic Study of Diabetes in the Elderly during 1996-2000

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[Abstract] Objective The aim was to determine the prevalence and incidence of type 2 diabetes mellitus (DM) and investigate the epidemiological characteristics as well as associated risk factors in the elderly people in Beijing. **Method** A survey was conducted among 2,239 subjects aged 60 to 90 who had health examination in PLA general hospital from 1996 to 2000. All the subjects investigated have been living in Beijing for at least 5 years. They were interviewed according to a structured questionnaire by physicians and trained assistants in department of endocrinology. Fasting plasma glucose (FPG) and postchallenge (steam-bread contained 80 g carbohydrate) plasma glucose (PPG) were measured. The subjects whose PPG were more than 7.2 mmol/L received a standard 75-g OGTT with 10-h overnight fast. DM and IGT were diagnosed according to criteria of WHO in 1985. The prevalence and incidence of type 2 DM between the WHO criteria and the revised new ADA diagnostic criteria was compared. **Result** Each subject received at least 3 times of full examination during this period. The follow-up rate of cohort population was more than 90%, average follow-up duration was 3.7 years. The prevalence of DM increased from 17.7% in 1996 to 28.7% in 2000 ($P<0.0001$) and it increased significantly with age ($P<0.001$). And the new ADA criteria failed to diagnose 75% of WHO diabetic patients. The prevalence of IGT increased from 14.0% in 1996 to 14.8% in 2000 ($P>0.05$). 234 subjects were identified as DM, the incidence for the elderly was 34.33 1000⁻¹ year⁻¹. And 232 subjects were identified as IGT, the incidence was 40.28 1000⁻¹ year⁻¹. 134 subjects of NGT at baseline had been converted to DM, the transformation rate was 8.8% in 4 years. 100 subjects of IGT at baseline had been converted to DM, the transformation rate was 35.8% in 4 years. The rate of transformation to DM was significantly higher in IGT group than that in NGT ($P<0.0001$). Logistical regression showed FPG, PPG, MAP (mean arterial pressure) and BMI were risk factors related to incidence of DM. **Conclusion** the prevalence and incidence of DM in the elderly was significantly higher than in general population. Because diabetic subjects associated with multiple metabolic disorders, the frequencies of cardiovascular disease were markedly higher in DM than those in non-DM population. We should emphasize prevention and treatment for DM as well as related cardiovascular risk factors. More attention should be paid to PPG in the early screening of DM.

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Analysis and Evaluation of Diabetes Mellitus in Elderly People by the
Application of the World Health Organization and American Diabetes
Association Diagnosis Criteria

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[Abstract] Objective To evaluate the sensitivity and specificity of the World Health Organization (WHO) criteria of DM and American Diabetes Association (ADA) criteria of DM when they are applied to elderly people, and to assess the use of fasting plasma glucose (FPG) alone for the screening of DM as defined by a 2-hour plasma glucose (PG 2h) ≥ 11.1 mmol/L following a 75-g oral glucose tolerance test (OGTT) and to determine the optimal FPG cut-point as well. **Method** We selected 1,234 subjects without a previous history of DM aged 60 to 90, and grouped them into different glucose levels by WHO or ADA criteria according to their standard 75-g OGTT results, and analyzed the concordance and the discordance between these populations. The WHO criteria of DM (PG 2h ≥ 11.1 mmol/L) was selected as the gold standard. We assessed the variations of sensitivity and specificity on ADA criteria of DM (FPG ≥ 7.0 mmol/L) and determined the optimal FPG cut-point in the elderly. **Results** The prevalence of DM was 3.16% and 16.28% by FBG criteria and PG-2h criteria respectively. The sensitivity of diagnosed DM was 15.3% and specificity was 99.2% according to ADA criteria. The coincidence percentage under the two criteria was only 15.3%. According to WHO criteria there were 196 newly diagnosed DM cases, however only 30 cases were diagnosed as DM by ADA criteria in all subjects. The coincidence percentage under IFG and IGT was only 4.5%. The coincidence percentage under NGT and NFO was 98.53%. The optimal FPG cut-point of diagnosed DM was 5.5 mmol/L in the elderly, which is affected by gender, age, body mass index (BMI) and the presence of hypertension. Using multiple linear regression analysis to compare the relation of FPG to PG2h in the overall subject group, the FPG corresponding to PG2h < 7.8 mmol/L was < 5.0 mmol/L. **Conclusion** There was lack of concordance between WHO and ADA criteria in the elderly. The ADA criteria could not replace for WHO criteria when it was used to diagnose DM patients. And the omission diagnostic rate of ADA criteria would have increased remarkably if it was applied to the elderly population aged ≥ 70 and BMI < 25 . We suggest that the elderly people with FPG ≥ 5.0 mmol/L should receive standard 75-g OGTT.

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FASTING PLASMA GLUCOSE IN NON-DIABETIC JAPANESE
WAS ELEVATED IN LATE 1990S

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Background and Aims: The life style in Japan has been dramatically changed since 1970s mainly because of the changes of eating habit in younger generation as well as those in many ethnics. This study is to investigate the changes of fasting plasma glucose (FPG), body mass index (BMI), serum total cholesterol, triglyceride, uric acid, blood pressure. **Materials and Methods:** 45,606 healthy subjects (aged 20-59 years, 14,443 women and 31,163 men) with FPG below 110 mg/dl were participated in 1994, 1995, 1997, 1998. ANOVA and lineal multiple regression analysis were used as statistical analysis. **Results:** FPG in women and men of every 10 year age groups was elevated significantly. FPG in women of 20s from 1994 to 1998 was 83.2(8.2)mg/dl (mean(SD)) to 87.7(6.8) ($p=0.0001$); of 30s from 1994 to 1998 was 86.5(7.1) to 88.7(6.8); of 40s from 1994 to 1998 was 88.3(7.4) to 91.1(7.4); of 50s from 1994 to 1998 was 89.7(7.9) to 92.8(7.8) ($p<0.0001$, respectively). FPG in men of 20s from 1994 to 1998 was 89.8(7.3) to 93.2(7.1); of 30s from 1994 to 1998 was 89.8(7.3) to 93.2(7.1); of 40s from 1994 to 1998 was 91.9(7.9) to 95.2(7.4); of 50s from 1994 to 1998 was 92.0(7.9) to 95.7(7.7) ($p<0.0001$, respectively). Lineal multiple regression analysis showed that BMI contributed to the changes of FPG in each age groups significantly ($p<0.001$). **Conclusions:** These findings indicate that FPG was elevated in healthy Japanese population in late 1990s. Multivariate analysis confirmed that the elevation of FPG was strongly dependent on BMI.

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Prevalence of Diabetes, according to ADA criteria, and associated risk factors in
a rural area of Baluchistan province

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Background and Aims: In other countries it has been observed that use of American Diabetes Association's (ADA) criteria shows a lower prevalence of abnormal glucose tolerance as compared to WHO criteria. In Pakistan almost all of the major studies have assessed the prevalence of abnormal glucose tolerance on the basis of WHO criteria only. This study was designed to assess the prevalence of abnormal glucose tolerance in a rural area of Baluchistan using the ADA criteria; and to assess the prevalence of, and association between selected risk factors for diabetes.

Materials and Methods: Sixteen randomly selected villages from the Lasbella district of Baluchistan were included in this study. All the households located in the selected areas were approached and all the family members aged 25 or more, who were available and willing to participate in the study were recruited. On the day of survey anthropometric and blood pressure measurements were taken; interviews were conducted to obtain demographic information and health history; and fasting blood samples were collected. The blood samples were transported to the laboratory and analysed using 'Good-Pap' Enzymatic Colourimetric test.

Results: A total of 670 males and 1362 females were studied. Prevalence rates for diabetes in males (10.1%) and females (4.3%) were only slightly lower than those studied done in similar areas of Pakistan using WHO criteria (10.2% males, 4.8% females). However the rates of IFG (4.2% males, 2.3% females) were much lower than the reported rates of IGT (7.4% males, 13% females).

Overall prevalence of obesity (22% vs 16%), hypertension (12.9% vs 6.5%) and positive family history for diabetes (1.6% vs 0.9%) was higher among diabetics as compared to non diabetics. However the difference was statistically significant (P at least <0.01 in each case) only among females.

Conclusion:

Appropriateness of using ADA criteria for screening population at risk of diabetes is questioned. Missing a large proportion of people with abnormal glucose tolerance would make prevention difficult. The results indicate need of continual use of OGTT for screening people at high risk of developing diabetes and perhaps also for diagnosing diabetes.

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The annual incidence rate of diabetes and diabetic complications: A population-
based study in Tayside, Scotland

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Background and Aims: To accurately calculate the incidence rates for diabetes and diabetic complications for the population of Tayside, Scotland: A St Vincent analysis.

Materials and Methods: The Diabetes Audit and Research in Tayside, Scotland (DARTS) register was used to identify Tayside patients diagnosed with type 1 or type 2 diabetes before or during the study period (Jan-Dec 1997). Multiple data sources were used to identify patients who developed a pre-defined diagnosis of a diabetic complication during this period. The DARTS register includes information from all primary care records extracted retrospectively by research nurses (available for 96% of patients), and data from all hospital admissions, all dispensed prescribing, biochemistry results, retinal screening and diabetes clinics. The rates of incident (i.e. first) microvascular and macrovascular complications were calculated for both type 1 and type 2 diabetes.

Results: In a population of 385,774 people, there were 917 and 6,613 patients with type 1 and type 2 diabetes and a further 22 patients diagnosed with type 1 and 672 patients diagnosed with type 2 diabetes in 1997, giving annual incidence rates of 5.82 per 100,000 persons and 177.66 per 100,000 persons respectively. The rates of diabetic complications per 1000 patients for type 1 and type 2 diabetes respectively were: Registered blindness 1.06, 3.84; End stage renal failure 3.19, 0.41; Lower limb amputation 2.13, 2.33; Peripheral vascular disease 4.26, 10.30; Angina 6.39, 15.93; Hypertension 17.04, 24.98; Myocardial infarction 4.26, 8.78; Stroke 4.26, 10.03.

Conclusions: These are the first United Kingdom data to give accurate incident rates of diabetes and of St Vincent complications. The low incident rates for microvascular complications in particular would make it difficult to detect reductions with statistical significance. Population-based definition of diabetes-related outcomes is possible using co-ordinated clinical information technology.

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Incidence of impaired fasting glucose, impaired glucose tolerance and type 2 diabetes in a German risk population: the RIAD study

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Background and Aims: Only scarce information exists about incidence of impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) in European risk populations for diabetes. The same applies for the contribution of IFG to the risk of subjects with IGT. We therefore investigated the incidence of these categories among participants of the Risk Factors in IGT for Atherosclerosis and Diabetes Study (RIAD).

Materials and Methods: At baseline a 75 g standard OGTT was performed in 1139 middle-aged subjects (40-70 years) at risk for the development of diabetes, such as family history of type 2 diabetes (55.1%), obesity (52.7%) and/or hyper- or dyslipoproteinemia (39.1%). Exclusion criteria were known diabetes and drugs affecting glucose tolerance. After an average follow-up time of 2.9 years in 548 subjects a second OGTT was performed. Plasma glucose was measured by the hexokinase method.

Results: We classified the participants by the results of the baseline OGTT according to WHO criteria in type 2 diabetes (DM2) (n=65), impaired glucose tolerance (IGT) (n=38), impaired fasting glucose (IFG) (n=85), in the combination IFG/IGT (n=35) and normal glucose tolerance (NGT) (n=325). Subjects with NGT at baseline showed a conversion rate to IFG of 1.3% per year, 3.9% to IGT, 0.5% to IFG/IGT and 0.6% to DM2. Participants with isolated IFG at baseline converted in 2.4 % per year to DM2. Subjects with isolated IGT had a yearly conversion rate to DM2 of 2.7% and those with baseline IFG/IGT combination changed in 9.9% per year to DM2.

Conclusions: Subjects on risk for diabetes by positive family history and/or diseases of the metabolic syndrome with NGT nevertheless have a high rate to convert to IFG, IGT or DM2. The combination IFG/IGT in the examined population has a four times higher rate to convert to diabetes than isolated IFG or IGT.

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Epidemiology – Type 2 Diabetes: Screening and Diagnostic Criteria

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Gender differences in the relationship between fasting and 2-hour glucose levels in Mauritius

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Background and Aims: It is already apparent that there are significant differences between IFG and IGT, both in their respective prevalences within a population, and in the classification of individuals. Furthermore, it would also appear that there might be some sex differences, with IGT being more common in women and IFG more common in men. This study compared the prevalence of various categories of glucose tolerance in the population of Mauritius stratified by sex and investigated possible explanations for the apparent gender differences.

Materials and Methods: A total of 6,098 individuals from the three main ethnic groups in Mauritius participated in a community-based cross-sectional survey in 1998. Categories of glucose metabolism were determined by an OGTT. Other cardiovascular risk factors were assessed.

Results: Diabetes prevalence and the prevalence of co-existing IFG with IGT were similar for males and females. Men were twice as likely to have isolated IFG than were women (4.1 vs 2.0%, respectively). Conversely, the prevalence of isolated IGT was significantly higher in women than men (12.1% vs 8.6%). Among non-diabetic individuals, the mean age-adjusted fasting glucose values were 5.2 and 5.1 mmol/l ($p<0.001$) in males and females respectively and the mean 2-hour glucose values were 5.9 and 6.5 mmol/l ($p<0.001$). For any given level of fasting plasma glucose the 2-hour plasma glucose was significantly higher in females than males. Even after adjustment for measures of obesity, lipids and insulin these differences remained.

Conclusions: In Mauritius the distribution of categories of impaired glucose metabolism differs by sex. This seems to be driven by a difference in the relationship between fasting and 2-hour glucose values in men compared to women. The observation that IFG is more prevalent in males and IGT more prevalent in females raises important questions about their underlying aetiology and the ability of the current glucose thresholds to identify high-risk categories equally amongst men and women.

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PROGNOSTIC VALUE OF FASTING AND POSTPRANDIAL GLYCAEMIA IN OFFSPRING OF PARENTS WITH CONJUGAL TYPE 2 DIABETES.

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Background and Aims: The offspring of parents with conjugal type 2 diabetes is particularly useful for comparing the prospective value of IGT according to the WHO-1985 criteria and IFG by the ADA-1997 guidelines. **Material and Methods:** In the group of 77 subjects (34 men and 43 women) aged 18-59 yrs (mean age 38 ± 0.8 [SEM]) whose both parents had type 2 diabetes, the OGTT was performed at a 5-year-interval, comprising the determination of glycaemia (whole venous blood) and serum insulin (RIA), as well as the measurement of fasting serum lipid profile. **Results:** In the preliminary assessment in 4 subjects a previously unknown diabetes was discovered and they were excluded from further study; 17 subjects had IGT and three out of those also IFG. The isolated IFG was diagnosed only in one person. Five years later, diabetes developed in 4 subjects (including one person with a previously diagnosed IFG), the number of subjects with IGT increased to 22, and IFG was diagnosed in 9 subjects in whom it was associated with the increase of 2h blood glucose of the OGTT. At that time, remission was observed in one person with IFG and in two subjects with IGT; the IGT person differed from those with normal glucose tolerance (31) by age (44.6 ± 1 vs 41.3 ± 1 yrs, $p=0.027$), fasting glycaemia (5.1 ± 0.12 vs 4.5 ± 0.10 mmol/l, $p=0.0004$), 2h serum insulin level of the OGTT (0.61 ± 0.09 vs 0.36 ± 0.05 nmol/l, $p=0.019$) and insulin resistance (HOMA IR scores: 2.59 ± 0.23 vs 2.07 ± 0.16 , $p=0.035$). It is noteworthy that the IGT subgroup with normal fasting blood glucose differed significantly from the IGT subgroup associated with IFG by higher 2h serum insulin level of the OGTT (0.71 ± 0.12 vs 0.34 ± 0.08 nmol/l, $p=0.039$) and a lower HDL Chol/Total ratio (19 ± 1 vs 23 ± 2 , $p=0.047$). **Conclusions:** In offspring of parents with conjugal type 2 diabetes IGT occurs earlier and more frequently than IFG and that the increased postprandial glycaemia is significantly associated with decreased insulin sensitivity.

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Withdrawn

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Fasting plasma glucose predicted but also undervalued type 2 diabetes incidence among high-risk Spanish individuals

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Background and Aims: Data on the predictive role of fasting (FPG) and postload (2hPG) non-diabetic hyperglycaemia with respect to diabetes incidence are reported by means of a prospective multicentre (10 primary health-care centres) cohort study.

Materials and Methods: Undiagnosed subjects aged >40 y. with one or more risk factors were screened according to the WHO rules (75g oral glucose tolerance test) to measure FPG and 2hPG. Two diabetes-free cohorts (n=243) with FPG<7.8mM and 2hPG<11.1mM were evaluated for 37.2 month (4.3-69.7) median follow-up. Impaired (IGT) and normal (NGT) glucose tolerance cohorts received an educational advice on dietetics and cardiovascular health. Index and probability of diagnostic variation (Kaplan-Meier and Cox proportional model) were assessed.

Results: IGT cohort (n=137) differed from NGT cohort (n=106) as for age (61.1/57.9; p<0.02) but neither for sex or BMI, nor for overall risk factor impact. At study close, 63 subjects (25.9%) developed diabetes, 43 with baseline IGT (31.4%) and 20 with NGT (18.9%). Mean annual incidence was 9.2% (14.2% for IGT and 5.3% for NGT). Using FPG as the main diagnostic criterion only 30 from 231 non-diabetic subjects (13%) developed the disease, 21 (38.2%) with impaired FPG (IFG) and 9 (5.1%) with normal FPG. Mean annual incidence would have been 4.6% (15.7% for IFG and 1.7% for normal FPG). Results from proportional hazards regression model indicated that hyperglycaemia was the most relevant predictive factor. Multivariate analysis evidenced no predictive value as for age, sex, BMI and classical risk factors.

The risk of developing diabetes at follow-up increased with increasing baseline FPG [OR=2.46 (1.78-3.40)] and 2hPG [OR=1.24 (1.08-1.42)]. Paradoxically, FPG interval that defines IFG category [6.1,<7] mM failed to predict diabetes [OR=1.29 (0.87-1.92)] compared to 2hPG interval [7.8,<11.1] mM that defines IGT category [OR=1.88 (1.41-2.51)].

Conclusions: FPG value surpassed 2hPG value as a predictor for the disease but also undervalued diabetes incidence. However, IGT diagnostic interval predicted diabetes incidence better than IFG interval. These findings suggest that a lower IFG limit (<6.1mM) would probably define a group at risk closer to the IGT group.

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IMPORTANCE OF TWO-HOUR PLASMA GLUCOSE IN OGTT

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Aims: This study was made to clarify necessity of OGTT using along-term follow-up observation. **Methods:** Subjects were a cohort of 20,685 persons who received OGTT in 1968 ~ 1998. **Results:** 1) Comparison of the trend of FPG and 2-h PG between DM development and control groups. FPG elevated from 12 years prior to onset, but the difference between the two groups is small, being almost within normal limits until one year before onset. 2-h PG was evidently high from 11 years prior to onset. As for the trend of insulin resistance, an evident increase was observed from 7-8 years prior to onset in obese group and gradually increased toward onset. In the non-obese group, it increased from 10-11 years prior to onset and continued until onset. 2) Effects of FPG or 2-h PG in OGTT on CHD mortality FPG or 2-h PG in OGTT were analyzed. FPG was classified into 4 groups, and furthermore each group was classified into two groups by 2-h PG <140 and ≥140 mg/dl. CHD mortality was 20~21/10,000 PY when 2-h PG was <140 mg/dl in all FPG groups. However, in the groups with 2-h PG ≥140 mg/dl in all FPG groups, the mortality was significantly elevated, indicating that CHD mortality is related to 2-h PG in OGTT. With elevation of 2h PG, CHD mortality gradually increased, but 170~199 group showed 1.8-fold increase in comparison with 140~169 mg/dl group. CHD mortality is related to 2h PG in IGT cases, but IFG was not a high-risk group. 3) Pulse wave velocity (PWV) is clinically employed for determining arteriosclerosis. The difference between each case and normal was expressed as ΔPWV. There was hardly any difference in ΔPWV when 2-h PG were <140, but from 140 mg/dl there was an evident increase and thereafter it showed a linear increase with 2-h PG. This suggests that with elevation of PG, arteriosclerosis is accelerated. It is demonstrated that ΔPWV is related to blood pressure, ΣIRI (fasting ~ 2-h), and TG. **Conclusion:** Even in the Japanese, IGT is a high-risk group of arteriosclerosis, but IFG is not. OGTT is very important for primary prevention of not only DM but also arteriosclerosis.

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IFG AND IGT: CLINICAL STAGES OR DIFFERENT PHYSIOPATHOLOGIC PHENOMENA?

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Background and Aims: The new classification of diabetes, released by American Diabetes Association and The World Health Organization, identified Impaired Fasting Glycemia (IFG) as fasting and Impaired Glucose Tolerance (IGT) as postprandial abnormalities of glucose regulation. Although a number of subjects classified as having either IFG or IGT by one parameter are normal by the other, the metabolic differences between IGT and IFG are not clearly established. The aim of our study was to identify some clinical and metabolic differences between IFG and IGT. **Materials and Methods:** In a group of seventy-five subjects with varying stages of abnormal glucose tolerance, investigated by a standard 75 g OGTT, twenty-one subjects who met the criteria for IGT or diabetes only by 2h plasma glucose values (group A: 17 IGT and 4 diabetes) and seventeen subjects who met the criteria for IFG or diabetes (group B: 13 IFG and 4 diabetes) only by fasting glucose values were identified. The differences in general characteristics and metabolic parameters between the two groups were evaluated. **Results:** There were no significant differences regarding mean (±sem) values for age (58.3±2.4 vs. 59.8±1.8 years), BMI (31.3±1.8 vs. 31.2±1.2), systolic (143.1±4.9 vs. 151.0±4.9 mm Hg) and diastolic (82.3±2.8 vs. 87.3±2.9 mm Hg) blood pressure between A and B group. The sum of plasma glucose values during OGTT was slightly greater in A group (26.4±0.7 vs. 24.9±0.5 mmol/L; p=0.097) but the difference was not significant. As compared to A, the B group was characterized by greater fasting glucose values (6.7±0.09 vs. 5.6±0.07 mmol/L; p<0.001). The A group had greater 2h OGTT (9.9±0.4 vs. 6.5±0.2 mmol/L; p<0.007) glucose values. The difference between groups in the mean increase of plasma glucose, above the basal levels, 1h after glucose load (5.0±0.4 vs. 5.4±0.4 mmol/L; p= 0.53) was not significant. The mean decrease of plasma glucose 2h after glucose load (vs. 1h values) was reduced (1.3±0.2 vs. 5.3±0.4 mmol/L; p<0.01) in A group. These data suggest that while a reduced clearance of the oral glucose load characterized group A, the increase in endogenous glucose production was predominant in B group. **In conclusion,** IFG and IGT may reflect different physiopathologic phenomena and not different stages of the same disease process.

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RISK FACTORS OF POSTTRANSPLANT DIABETES MELLITUS FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION

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Background and Aims: To investigate the clinical characteristics and possible risk factors for posttransplant diabetes mellitus (PTDM) following allogeneic bone marrow transplantation (BMT)

Materials and Methods: A total of 490 patients received allogeneic BMT at St. Mary's hospital during 1993-1999. We included that patients whose fasting glucose level was above 7.8 mmol/l at least 3 times and required insulin treatment to avoid the transient glucose intolerance due to steroid use. Age, sex, body mass index, mean daily steroid dosage, mean daily cyclosporin dosage, incidence of graft versus host disease (GVHD), incidence of cytomegalovirus (CMV) disease, fasting plasma glucose concentration, serum lipid profiles, and HLA phenotypes were retrospectively examined in 15 PTDM patients and 68 non-diabetic patients following allogeneic BMT.

Results: Among 490 allogeneic BMT, PTDM developed in 15 patients (3.1%). The mean duration from BMT to onset of PTDM was 26.6 \pm 33.9 days. Mean daily steroid dosage, incidence of GVHD, and incidence of CMV disease were significantly higher in PTDM group [55.9 \pm 32.9 vs 22.5 \pm 21.2 mg ($p=0.002$), 53.5 vs 11.8% ($p=0.001$), 33.3 vs 3.8% ($p=0.004$), respectively]. Among HLA phenotypes, the allele frequency of HLA-DR52 and DR53 were observed only in PTDM patients. At the onset of PTDM, fasting serum glucose, total cholesterol, and LDL-cholesterol concentration were significantly elevated than those observed in pre-BMT state [11.95 \pm 3.23 vs 5.73 \pm 0.62 mmol/l ($p<0.001$), 5.03 \pm 1.48 vs 3.73 \pm 1.01 mmol/l ($p=0.013$), 2.58 \pm 1.25 vs 1.55 \pm 0.79 mmol/l ($p=0.040$).

Conclusions: We conclude that PTDM following BMT frequently develops in patients with high-dose steroid treatment, occurrence of GVHD, and HLA-DR52 and DR53 phenotypes. This study suggested that high-dose steroid therapy, mainly due to GVHD, might be the critical factor in the onset of PTDM following allogeneic BMT and that the risk may be affected by HLA-DR52 and DR53 phenotypes.

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Epidemiology – Type 2 Diabetes: Metabolic Syndrome

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Prevalence of Glucose Intolerance/Diabetes Mellitus (ADA CRITERIA 1997), Obesity and Related Metabolic Disturbances in Spanish Population.

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Background and Aims: To investigate the relationships between Glucose Tolerance, obesity related anthropometric parameters: Body Mass Index (BMI), Sagittal Abdominal Diameter (SAD), Waist/Hip ratio (W/H), fasting insulin (FSI) and 2 hours (Ins 2hrs) post-glucose, fasting proinsulin (Prol) and 2hrs (Prol2hrs), fasting Leptin (Lep) and 2hrs (Lep2hrs). Insulin Resistance (IR), lipid profile, and blood pressure measurements.

Material and Methods: 2929 individuals recruited from nine Communities Nationwide, aged 35 – 64 yrs. (Females (F): 54.7%, Males (M): 45.3%). Type 1 DM were excluded. A) Anthropometric measurements: BMI, SAD, W/H ratio. B) Laboratory: parameters assayed: Glucose Tolerance Test (75gr.), Lipid profile [Cholesterol (CT), HDL-Cholesterol (HDL-C), Triglycerides (TG)], FSI (mU/ml), Ins 2hs (mU/ml), Prol (pM/l) and Lep (ng/ml) (measured by LINCOR RIA). Insulin Resistance (IR) estimated as FSI > fourth quartile in the general population. Statistical analysis: Student's test, ANOVA and multivariate logistic regression analysis (MLRA).

Results: 1) Glucose Tolerance: Diabetes Mellitus (DM) 7.1%, Impaired Glucose Tolerance (IGT) 7.0%, Impaired Fasting Glucose (IFG) 6.0%. 2) Obesity related parameters: mean BMI 27.86 (\pm 4.44), prevalence obesity (BMI \geq 30): 31.9%, SAD 22.46 (\pm 3.85), W/H was 0.94 (\pm 0.07). 3) Insulin, Proinsulin, Leptin: mean values FSI 13 (\pm 10) mU/ml, Ins 2hs 28 (\pm 27) mU/ml, Prol 12 (\pm 14) pM/l, Lep 12 (\pm 11) ng/ml. 4) Lipid profile: CT 222 (\pm 42) mg/dl, HDL-C 51 (\pm 13) mg/dl, TG 120 (\pm 85) mg/dl. 5) Blood pressure: systolic (SBP) 128 (\pm 21) mmHg, diastolic (DBP) 80 (\pm 12) mmHg. A BMI and SAD positive association with FSI, Prol and Lep were found. Obese subjects presented 34% more IFG and 46% more DM regardless SAD, sex, age. Individuals with Insulin Resistance (IR) presented 79% more IFG, 53% more IGT and 63% more DM. M presented 41% more DM than F. Subjects with IR presented higher leptin levels (43%) as compared to non IR.

Conclusions: Prevalence by ADA CRITERIA in Spanish population was IFG 6.0%, IGT 7.0% and DM 7.1%. Individuals with IR presented a higher atherogenic risk estimated by lipid profile in IFG and DM as compared to non IR. The prevalence of IFG and IGT was higher in individuals \geq 45 yr. Obese subjects presented higher prevalence of DM. In IR subjects the prevalence of DM was 48.4%, 41.2% IGT, 44.5% IFG; likewise individuals with IR had higher blood pressure values.

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Diabetes Mellitus and Idiopathic Haemochromatosis - A Prospective Study

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Background and Aims: Diabetes Mellitus (DM) and Idiopathic Haemochromatosis (IH) have long been associated. Sheldon (1935) and Niederau (1986) in a large series of patients with IH recorded DM in 80% and 54% of cases respectively. Others propose that DM in IH may be associated with insulin resistance and infrequent complications and that iron depletion leads to reduced insulin requirement. Because early diagnosis of IH is now common, resulting in changing clinical features, we have prospectively studied a group of patients with IH and DM to see if above observations are still valid.

Materials and Methods: 150 patients with IH were followed up for 17 years.

Results: 38 (25.3%) suffered from DM; mean age at diagnosis of DM was 62 years; mean age of duration of DM is 4 years. 22.8% have hepatic cirrhosis. In 34 cases (89.5%) the diagnosis of DM preceded or coincided with that of IH. 13 patients treated with diet, 15 with oral agents, 5 with insulin and oral agents and 5 on insulin alone. Iron depletion was followed by reduced insulin requirement in only 2 cases. Retinopathy occurred in 37%, neuropathy in 24%, proteinuria in 24%. Macrovascular disease was common; ischemic heart disease 18%, peripheral vascular disease 18%, cerebrovascular disease 11%, hypertension 55%.

Conclusion: Our observations suggest that DM occurs much less frequently in IH than previously recorded. Both macrovascular and microvascular disease are frequent complications and iron depletion rarely affects management of DM.

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PREVALENCES OF OBESITY AND CENTRAL OBESITY IN A CANARIAN COMMUNITY: ASSOCIATION WITH TYPE 2 DIABETES MELLITUS.

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Background and Aims: In our community the prevalence of type 2 diabetes mellitus is 19.7%, among the highest ever reported in Caucasian populations. Obesity, especially central obesity, is a paramount risk factor for type 2 diabetes mellitus. We sought to estimate the prevalences of obesity and central obesity and their association with type 2 diabetes mellitus in our community.

Materials and Methods: A random sample of 900 subjects over 30 years old (stratified by age and sex) was studied: data on age, sex and medication use were obtained, height, weight and waist perimeter were measured and standard oral glucose tolerance tests were performed. Subjects with a body mass index above 30 kg/m² were considered obese. Women with a waist perimeter above 88 cm and men with a waist perimeter above 102 cm were considered centrally obese.

Results: A total of 691 subjects were studied. The mean response rate was 76.8%. The response rate was similar in all age and sex groups. The prevalences of obesity/central obesity were 36.5%/66.5% in women and 23.6%/32.0% in men. These prevalences are among the highest in Europe. Bivariate analyses show a strong association of both obesity and central obesity with type 2 diabetes mellitus ($p<0.001$), but in a multivariate model, waist perimeter ($p<0.001$) but not body mass index ($p=0.212$) was retained as an independent predictor of type 2 diabetes mellitus.

Conclusions: The prevalences of obesity, central obesity and type 2 diabetes mellitus in our community are extremely high. Although both obesity and central obesity are associated with type 2 diabetes mellitus, central obesity is a better predictor of type 2 diabetes mellitus than obesity.

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NATIONAL DIABETES PREVENTION PROGRAMME IN POLAND: HIGH PREVALENCE OF METABOLIC DISORDERS IN URBAN POPULATION

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Background and Aims: In 1997-2000 a part of the National Diabetes Prevention Programme, an epidemiological study on type 2 diabetes, was conducted in the Central District of Lodz, Poland. The aim of this study was to assess the prevalence of type 2 diabetes and related metabolic disorders in a Central European urban population, in the context of the rising diabetes incidence worldwide. **Materials and Methods:** 6000 randomly chosen inhabitants of the Lodz Central District aged >35 years were invited by mail to participate in the study. In all subjects a personal and family history of diabetes, obesity, and cardiovascular disease were taken. Body weight, height, waist and hip circumferences were measured. Fasting blood glucose and lipid profile were assessed. In non-diabetes subjects an oral glucose tolerance test (75 g) according to the WHO protocol (OGTT) was performed. **Results:** 2018 persons (1217 [60.3%] female and 801 [39.7%] men) took part in the study, mean age 58.2 yrs (response rate 33.6%). 179 (8.9%) subjects had already diabetes diagnosed. In non-diabetes group fasting plasma glucose >7.0 and <7.8 mmol/l was found in 68 (3.4%) persons, and ≥7.8 (2.6%) mmol/l – in 53 subjects. OGTT was performed in 1574 (78%) subjects. 342 (16.9%) of them had impaired glucose tolerance (IGT) and 138 (6.8%) were diagnosed as having diabetes, which yielded the total prevalence of diabetes 15.7%. 108 out of 134 (78.3%) newly diagnosed diabetics had fasting plasma glucose <7.0 mmol/l. Body mass index ≥25 kg/m² was noted in 1432 (71%) subjects, and ≥30 kg/m² – in 626 (31%) persons. Total cholesterol ≥5.2 mmol was found in 1170 (58%) subjects and triglycerides ≥1.7 mmol/l – in 1392 (69%). **Conclusions:** The prevalence of metabolic disorders in a Central European urban adult population is strikingly high. Almost three out of four subjects had excessive body weight, and this phenomenon was associated with concomitant high rate of diabetes and lipid disorders. As the rate of the previously undiagnosed diabetes was higher than that of known cases, active attitude towards diabetes screening in general population is strongly recommended, with the preferred use of OGTT. In general, exceptionally high prevalence of metabolic disturbances in urban population calls for a diabetes-like national metabolic disorders prevention programme.

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PREVALENCE OF COMPONENTS OF INSULIN RESISTANCE SYNDROME IN SUBJECTS WITH ARTERIAL HYPERTENSION-POPULATION BASED STUDY.

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Assumption: Insulin resistance syndrome consists of several interconnected elements: disturbances of glucose and lipid metabolism, tendency to thrombosis, abnormal concentration of uric acid. The number and intensity of syndrome components are variable. **Aim:** Estimation and analysis in the representative group of hypertensive subjects selected from the defined population of the prevalence of: glucose abnormalities according to ADA classification; insulin levels > 15 mIU/l, hypertriglyceridemia >1,61 mmol/l; levels of HDL <1,0 mmol/l; LDL >4,14 mmol/l. Two subgroups: persons with and without arterial hypertension were statistically compared. **Groups under study:** The whole cohort under study was composed of 833 cases aged over 45; 44,4% of them were normotensive and 55,6% hypertensive. **Results:** In the hypertensive versus normotensive subgroup significantly higher prevalence of glucose metabolism disturbances were found: increased fasting glycemia 2,2% vs. 1,1%; impaired glucose tolerance 11,3% vs. 5,6%; diabetes mellitus type 2 10,4% vs. 7,0% (p<0,05). Comparison of hypertensive and normotensive groups revealed also significant differences in serum triglycerides >1,6 mmol/l-39% vs 29,4% (p<0,05); LDL cholesterol >4,1 mmol/l-40,4% vs 31% (p<0,05); HDL cholesterol <1,0 mmol/l 14,7% vs 13,2% (p>0,05); serum fasting insulin levels >15 mIU/l 12,8% vs 11,3% (p>0,05). **Conclusions:** Persons with hypertension have significantly higher prevalence of glucose metabolism abnormalities –present in 23,8%; of triglycerides > 1,61 mmol/l-present in 39,0%; of LDL >4,14 mmol/l-present in 40,4% as compared to normotensive persons. There were no important differences between analyzed subgroups frequencies of HDL< 1,0 mmol/l and insulin levels>15,0 mIU/l. On this basis the need for special metabolic approach in the hypertensive subjects should be considered.

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Insulin resistance is accompanied by increased von Willebrand factor levels in non-diabetic women: A study of offspring of Type 2 diabetic subjects compared to offspring of non-diabetic subjects.

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Background and Aims: Von Willebrand factor (vWF) and fibrinogen are haemostatic factors associated to the increased risk of cardiovascular disease seen in diabetes. The aims were to examine whether levels of von Willebrand factor (vWF), fibrinogen are related to a parental history of Type 2 diabetes and to determine possible explanatory factors for high versus low vWF and fibrinogen.

Materials and Methods: In this cross-sectional study we compared plasma vWF and fibrinogen in 88 non-diabetic offspring of Type 2 diabetic subjects (relatives) and 103 offspring of non-diabetic subjects (controls). With multiple logistic regression we determined explanatory factors for high versus low vWF and fibrinogen. An estimate of insulin resistance was calculated using the homeostasis model assessment (HOMA model).

Results: There were no significant differences in vWF (1.12 IU/ml vs. 1.06 IU/ml, p=0.296) or fibrinogen (3.2 g/l vs. 3.1 g/l, p=0.263) between relatives and controls. Age (p<0.01), urinary albumin excretion rate (p<0.05), ischaemic heart disease (IHD) (p<0.05) were found to be significant explanatory factors for vWF above the median (1.10 IU/ml). Interaction between insulin resistance (HOMA model) and sex was found. Odds ratio for high versus low insulin resistance was 18.39 (p<0.001) for women and 1.92 (p=0.32) for men. BMI (p<0.05), sex (p<0.01), smoking status (p<0.05) and IHD (p<0.01) were significant explanatory factors for fibrinogen above the median (3.1 g/l).

Conclusions: Levels of vWF and fibrinogen were not influenced by a parental history of Type 2 diabetes. Insulin resistance was found to be a significant risk indicator for high vWF only in women. This may indicate that insulin resistance is a higher risk factor for women than for men, when the outcome is endothelial dysfunction possibly resulting in overt cardiovascular disease.

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INSULIN RESISTANCE AND HEMOSTATIC FACTORS: FREQUENCY AND DISTRIBUTION IN A WELL DEFINED POPULATION

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Assumptions. Insulin resistance in the presence or absence of hyperglycemia, acts as independent cardiovascular risk factor. One may assume, that one of the possible links underlying this phenomenon is the activation of the atherogenic procoagulatory state.

Aims. For this reason the study of insulin resistance in the well defined population was undertaken. The aim was to determine frequency and distribution of insulin resistance, hemostatic factors and to examine correlations between these variables. The sample of 269 subjects was selected by the demographic, proportional method representing therefore the natural population structure. Standardised clinical examination was performed in all cases.

Methods. Laboratory tests included determination of blood glucose (glucose oxidase method), insulin (IRMA, Polatom), C-peptide (Biodata), fibrinogen (Biopol), t-PA (Immulyse-t-PA, Biopol), PAI-1 (Biopol). Insulin resistance was assessed by the HOMA Index supported by Berglunds Insulin Sensitivity Index.

Results. HOMA index characteristic for insulin resistance was found in 26% and Berglunds Index in 6.3% of cases. Fasting hyperinsulinemia was present in 15.2% of persons under study. The mean values for fibrinogen was 241.4 ± 50.8 mg/dl, for t-PA 8.71 ± 6.31 ng/ml, for PAI-1 11.66 ± 9.58 ng/ml, for PAI-1 activity 12.03 ± 9.33 U/ml. Mean values of fibrinogen, t-PA and PAI-1 serum concentration and activity were significantly higher in the subgroup with insulin resistance, than in general population. Statistically valid negative correlations between Insulin Sensitivity Index and t-PA and PAI-1 were found in whole population. Statistically significant correlation between HOMA Index and PAI-1 and t-PA was also found.

Conclusions. Correlation between insulin resistance and hemostatic factors as found in the defined population represent the important mechanism in the pathogenesis of atherosclerosis. Its early diagnosis may serve as the basis for intervention.

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Obesity, body fat distribution and hyperinsulinaemia in the development of multiple metabolic syndrome in the San Antonio Heart Study.
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Background and Aims: Using body mass index (BMI), waist circumference, waist to hip ratio and insulin levels to predict the development of metabolic disorders which include dyslipidemia (triglycerides ≥ 2.26 mmol/l or HDL cholesterol ≤ 0.91 mmol/l in men, ≤ 1.17 mmol/l in women), hypertension (systolic or diastolic blood pressure ≥ 140 or ≥ 90 , respectively), and type 2 diabetes (fasting or two-hour test glucose ≥ 7.0 or ≥ 11.1 mmol/l respectively, or medications for diabetes).

Materials and Methods: Seven year longitudinal study of 246 male and 301 female non-Hispanic Whites and 435 male and 660 female Mexican Americans, aged 25 to 64 years, from the second cohort of the San Antonio Heart Study, Texas.

Results: Adjusting for age, ethnicity and lifestyle, the risks of developing multiple metabolic syndrome (any two or more of the metabolic disorders) were 3.7 (95% confidence interval: 2.0 to 6.7) in men and 8.3 (4.7 to 14.6) in women with BMI ≥ 30 kg/m² compared to those with BMI < 25 kg/m². Compared to men and women with waist circumferences below 94 cm and 80 cm, respectively, men with waist ≥ 102 cm were 2.8 (1.6 to 4.9) times and women with waist ≥ 88 cm were 5.9 (3.6 to 9.8) times more likely to develop the syndrome. People with high waist to hip ratio and insulin levels were also at significant risk of developing metabolic disorders. High anthropometric indices remained significant predictors of metabolic disorders after additional insulin adjustment. After seven years, 33% of subjects who had a combination of high BMI (≥ 30 kg/m²) and high waist circumference (≥ 102 cm in men and ≥ 88 cm in women) developed multiple metabolic syndrome, compared to 20% of subjects with either a high BMI or a high waist, and 10% of subjects with both low BMI and low waist.

Conclusions: These findings support the National Institute of Health recommendation for weight management in people with high body mass index and large waist circumference, who should seek professional help to reduce the high risk of developing multiple metabolic syndrome. Adjustment for baseline fasting insulin levels had only a modest effect on anthropometry in the prediction of the incidence of the syndrome.

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The Metabolic Syndrome among the Inuit of Greenland

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Background and Aims: Compared to Denmark the incidence of cardiovascular disease and diabetes is low among the Inuit of Greenland. The Greenlandic society is currently undergoing a rapid transition. The Inuit of Greenland is a population with a probable genetic potential for developing high incidences of cardiovascular diseases and diabetes, but in contrast with populations like the Pima and other American Indians and the Alaska Inuit they have retained a life style that counterbalances the genetically high risk for disease. The aim of the present study is to compare the prevalence of components of the metabolic syndrome, including hypertension, abnormal glucose metabolism, dyslipidemia, microalbuminuria, central obesity, and overall obesity, between Inuit living in Greenlandic villages, and in Greenlandic towns. **Materials and Methods:** From 1999 to 2001 a total of 976 individuals aged 35 and over participated in a health examination. A random sample of indigenous Greenlanders was selected from Nuuk (capital), Qassigiannuit (a small town on the West coast), while all adult inhabitants in four selected villages in Uummannaq district were invited to participate. Diabetes and impaired glucose tolerance were diagnosed using the oral glucose tolerance test. BMI, waist-to-hip ratio, and blood pressure were measured, and blood and urine samples were taken from each subject. Sociodemographic characteristics were investigated using a questionnaire. **Results:** Hypertriglyceridemia, low HDL cholesterol, overall obesity, and hypertension were significantly more prevalent in towns in Greenland, whereas central obesity was more prevalent in the settlement population. Prevalence of elevated albumine/creatinine ratio was not significantly different between the populations. The age-adjusted prevalence of the metabolic syndrome as defined by the World Health Organization was higher in the towns (Nuuk 18.3%, Qassigiannuit 16.2%, Uummannaq 13.7%, $p < 0.05$), but diabetes was more common in the settlements (Nuuk 7.7%, Qassigiannuit 10.2%, Uummannaq 11.2%, $p < 0.05$, age-adjusted). **Conclusions:** Despite the high prevalence of the metabolic syndrome in the most westernised parts of Greenland, diabetes was more common in the settlements. Further analysis and adjustment for confounders is demanded to confirm and explain the results.

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Epidemiology – Type 2 Diabetes: Outcomes

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SURVIVAL IN PATIENTS WITH TYPE 2 DIABETES IN A SWEDISH COMMUNITY

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Background and Aims: To explore risk factors for all cause mortality in patients with type 2 diabetes treated in primary care. **Materials and Methods:** A prospective population-based study of 400 patients with type 2 diabetes who consecutively completed an annual check up during 1992-1993. Vital status was followed to year 2000. Associations between baseline characteristics and survival were analysed by Cox regression with adjustment for age and gender and were expressed as relative risk (RR) with 95 per cent confidence interval (CI), by 1 SD for continuous variables. **Results:** In both genders, patterns were similar and both men and women were thus analysed together. All cause mortality was associated with elevated HbA1c levels (RR = 1.2 CI; 1.02-1.4), serum triglycerides (RR = 1.2 CI; 1.03-1.4) and with low-density lipoprotein cholesterol/high density lipoprotein (LDL/HDL) ratios (RR = 1.2 CI; 1.0-1.4). An increased mortality risk was seen also with co-morbidity such as hypertension (RR = 1.72 CI; 1.21-2.46), microalbuminuria (RR = 1.87 CI; 1.27-2.76) and previous cardiovascular disease (RR = 1.7 CI; 1.15-2.50). Subanalyses revealed that increased mortality related to HbA1c was restricted to hypertensive patients with type 2 diabetes (RR = 1.3 CI; 1.05-1.7). **Conclusions:** Potentially modifiable risk factors for all cause mortality in patients with type 2 diabetes included impaired glucose and lipid metabolism and hypertension. Patients with hypertension in addition to type 2 diabetes may be more susceptible to hyperglycaemia than normotensives. Patients with microalbuminuria or with previous cardiovascular disease are also at particularly high mortality risk.

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Decreased mortality associated with the use of metformin compared to sulfonylurea monotherapy in type 2 diabetes

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Background and Aims: Metformin, alone or in combination, is effective in reducing hyperglycemia and microvascular complications in type 2 diabetes. However, questions remain regarding the impact of metformin therapy on macrovascular complications and cardiovascular mortality. The aim of this study was to examine the relationship between metformin and sulfonylurea (SU) use and mortality in new users of these agents.

Materials and Methods: Saskatchewan Health databases were used to examine population-based mortality rates for new users of oral antidiabetic agents. Individuals with prescriptions for SU or metformin in 1991 to 1996 and no use in the year prior were identified as 'new users'. Prescription records were prospectively followed for 1 to 9 years. Cardiovascular-related deaths were identified based on ICD-9 codes for underlying cause recorded on death certificates. Multivariate logistic regression analyses were used to assess the differences in mortality between drug cohorts, after adjusting for potential confounding variables.

Results: The study sample comprised 12,272 new users of oral antidiabetic agents, with an average of 5.1 (SD 2.0) years of follow-up. Among those subjects with at least one year of drug exposure, mortality rates were 750/3,033 (24.7%) for subjects receiving SU monotherapy, 159/1,150 (13.8%) for metformin monotherapy, and 635/4,683 (13.6%) for combination therapy. After adjusting for age, sex, comorbidity, and the presence of established coronary disease, metformin monotherapy was associated with reduced risk for all-cause (Odds Ratio [OR] 0.60, 95% CI 0.49-0.74) and cardiovascular (OR 0.65; 0.50-0.86) mortality compared to SU monotherapy. SU plus metformin combination therapy was also associated with reduced all-cause (OR 0.66; 0.58-0.75) and cardiovascular (OR 0.65; 0.54-0.77) mortality.

Conclusions: After adjusting for factors associated with increased cardiovascular risk, we found that metformin, alone or in combination with SU, was associated with reduced all-cause and cardiovascular mortality compared to SU monotherapy among new users of these agents.

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RELATIONSHIP OF PHYSICAL ACTIVITY TO MORTALITY AMONG ADULTS WITH DIABETES IN THE UNITED STATES

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Background and Aims: Physical activity is associated with longevity, but few studies have examined this relationship among people with diabetes. We examined whether higher levels of walking and recreational physical activity are associated with a lower risk of all-cause and cardiovascular disease (CVD) mortality among adults with diabetes in the United States. **Materials and Methods:** Physical activity, demographic factors and comorbidities were assessed for 2739 men and women with self-reported diabetes aged ≥ 35 years as part of the 1990 and 1991 National Health Interview Surveys. We categorized individuals according to the number of hours per week spent walking, doing any recreational physical activity, or moderately vigorous activity. Deaths were ascertained using a National Death Index search for a 6 year period, during which there were 478 deaths due to all-causes (crude risk = 3.9% per year) and 242 CVD deaths (2.0% per year). **Results:** Higher levels of walking, total recreational physical activity, and moderately-vigorous physical activity were each associated with reductions in all-cause mortality. Compared to inactive individuals, those who reported walking at least 2 hours per week had a 41% reduction in all cause mortality risk (RR=0.59, 0.42 to 0.83), controlled for age, race, smoking, body mass index (BMI), and comorbidities. Similar associations were present for participation in at least 2 hours of total physical activity (RR=0.66, 0.49 to 0.89), and moderately-vigorous activities (RR=0.64, 95% CI, 0.45 to 0.92). Higher levels of physical activity also tended to be associated with reductions in heart disease mortality (RR=0.69; 0.45 to 1.05 for adults walking at least 2 hours per week). Walking was more protective against all-cause mortality among non-insulin users (RR=0.54, 0.71 to 1.01) than among insulin users (RR=0.85 95% CI, 0.45 – 1.58). The protective association of physical activity was observed across sex, race, BMI, age and diabetes duration groups. **Conclusions:** Walking and recreational physical activity are likely to enhance longevity across a diverse spectrum of adults with diabetes.

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Mortality and Abnormal Glucose Tolerance States in Central Lancashire- A Seven year Follow-up Study

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Background and Aims: To examine the link between abnormal glucose tolerance states and mortality.

Materials and Methods: 2519 people referred with suspected diabetes for a glucose tolerance test were followed up for between 3 and 7 years. There were 395 people with newly diagnosed diabetes of whom 100 had isolated post challenge hyperglycaemia (IPH), 28 isolated fasting hyperglycaemia (IFH) and 198 combined hyperglycaemia. There were 376 people with impaired glucose homeostasis of whom 265 had impaired glucose tolerance (IGT) and 111 with impaired fasting glycaemia (IFG).

Results: Compared with people without diabetes, people with IPH had an increased all cause mortality ($P<0.0001$) and of cardiovascular mortality ($P<0.0001$) similar to those with combined hyperglycaemia(CH). Those with IFH had an increased risk of all cause mortality ($P<0.0001$) and to a lesser extent cardiovascular mortality ($p=0.004$). People with IGT had an increased risk of all cause mortality ($P<0.0001$) but not that of cardiovascular mortality ($p=0.06$), while IFG had no significant difference in mortality compared with people without diabetes.

Conclusion: These data highlights the strong association of abnormal glucose tolerance states and mortality including IPH and IGT which can only be identified by the performance of a glucose tolerance test.

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LOW IRS 1 PROTEIN EXPRESSION IS ASSOCIATED WITH A DISORDERED METABOLIC STATE AND AN INCREASED INTIMA-MEDIA THICKNESS IN THE CAROTID ARTERY

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Background and Aims: Low IRS 1 protein expression in fat cells can identify individuals at risk for type 2 diabetes. To further characterize the metabolic phenotype in subjects with low IRS 1 protein expression, we assessed the metabolic profile in conjunction with surrogate markers for atherosclerosis.

Materials and Methods: We recruited 45 male healthy first-degree relatives of type 2 diabetes patients and 40 male healthy non-diabetic control subjects. They were investigated with an intravenous glucose tolerance test and ultrasound measurements of the right carotid artery. All subjects were asked to undergo a subcutaneous needle biopsy and 30 subjects were willing to participate. The protein content of IRS 1 was analyzed by immunoblotting and a reduction of $>50\%$ as compared to control subjects was required for the definition low IRS 1 protein content. We identified 11/26 subjects with marked reduction in adipocyte IRS 1 content (LOW), 9/26 with normal IRS 1 content (CON), and 6/26 subjects were not typical, and therefore not included in the analysis.

Results: Age (45 ± 3 vs 42 ± 2 yrs (Mean \pm SE), BMI (26.8 ± 0.5 vs 25.7 ± 0.9 kg/m²), blood pressure and nicotine consumption was similar in LOW and CON, respectively, whereas W/H-ratio was increased in the LOW group (0.95 ± 0.01 vs 0.90 ± 0.02 , $p=0.048$). Insulin sensitivity was decreased (224 ± 90 vs 407 ± 72 ml/mU/min, $p=0.044$), acute insulin response (0-10 min) enhanced (613 ± 128 vs 258 ± 51 mU/l, $p=0.030$) and fasting proinsulin higher (16.9 ± 2.5 vs 8.5 ± 1.1 pmol/l, $p=0.017$) in the LOW group. Moreover, intima-media thickness (IMT) in the right carotid bulb was increased in the LOW group (0.949 ± 0.080 vs 0.642 ± 0.044 mm, $p=0.008$), but no differences appeared in the lipid profile or cellular adhesion molecules in the groups.

Conclusions: Low IRS 1 protein expression is associated with risk for type 2 diabetes and, as shown, an increased IMT in the carotid artery. Thus, low IRS 1 protein content may precede both type 2 diabetes and atherosclerotic vascular disease.

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Cardiovascular risk profile in early diagnosed type 2 diabetes: preliminary results from a two step screening procedure

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Background and aims: Little is known about the characteristics of patients identified by screening compared to routinely diagnosed diabetic patients. In this study we compared the cardiovascular risk profile of screening detected diabetes with that of routinely diagnosed patients.

Methods and materials: A two step screening procedure was performed, consisting of the Symptom Risk Questionnaire and a fasting capillary glucose measurement. In subjects with a capillary glucose of > 5.5 mmol/l an OGTT was performed (screening detected diabetes mellitus (SDM). The control group consisted of consecutive new diabetic patients diagnosed by general practitioners (GPDm). In all newly detected diabetic patients (both SDM and GPDm) cardiovascular risk factors were assessed.

Results: In the first part of the screening 91 patients were detected, in 80 (88%) additional measurements were performed. The general practitioners identified 39 new patients with type 2 diabetes. Mean age was $63.4 (\pm 6.3)$ for SDM and $62.3 (\pm 6.4)$ for GPDm subjects. Fasting plasma glucose and HbA1c were lower in the SDM subjects: FPG $7.56 (1.51)$ vs $9.53 (2.75)$ mmol/l, HbA1c $6.29\% (1.24\%)$ and $8.70\% (2.26\%)$. In comparison with GPDm subjects, hypertension, high LDL, high total cholesterol and microalbuminuria were more common in SDM subjects: (HT 57.5% vs. 45.7% ; LDL 81.8% vs. 65.8% ; TCHOL 76.3% vs. 64.1% ; MA 36.8% vs. 30.4%). The percentage of subjects with high triglyceride levels was similar in both groups. Low HDL cholesterol was more common in GPDm patients and this finding was most prominent in women.

Conclusions: This screening procedure identifies subjects with previously unknown diabetes with relatively mild hyperglycaemia, but characterised by an adverse cardiovascular risk profile. The latter finding may be inherent to the screening procedure.

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Recent trends in cardiovascular mortality of diabetic patients in England and Wales
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Background and Aims: Mortality statistics can be used as a way to monitor the effectiveness of programmes directed at reducing mortality from cardiovascular disease in diabetic patients. Mortality data from the Office of National Statistics for 1975-6, 1985-6 and 1995-6 was used to examine trends in diabetes related mortality in England and Wales.

Materials and Methods: Data was extracted from all death certificates mentioning diabetes for 1975-6, 1985-6 and 1995-6. Rates of mentioning diabetes per 100,000 population were calculated for nine age groups and standardised by age to the average of the mid-year populations in 1975-6 to produce directly standardised rate ratios.

Results: In women, between 1975-6 and 1985-6 there was an age standardised decrease of 11.7% (10.6,12.8) in deaths in which diabetes was mentioned on the certificate and a further fall in 1995-6 of 20.7% (19.1,22.3) standardised to 1975-6. In men there was a small increase in 1985-6 of 2.7% (1.4,4.1) in deaths in which diabetes was mentioned on the certificate and a small fall of -1.8% (-3.9,+0.2) in 1995-6, standardised to 1975-6. Deaths with diabetes mentioned on the certificate which had ischaemic heart disease (IHD) as the underlying cause had a small age standardised decrease in women between 1975-6 and 1985-6 of -1.8% (-4.1,+0.6) and a larger fall of 10.6% (7.3,13.9) in 1995-6, standardised to 1975-6. In men, between 1975-6 and 1985-6 there was an age standardised increase of 14.6% (11.5,16.8) in IHD deaths with diabetes on the death certificate. In 1995-6 the increase in male IHD deaths standardised to 1975-6 was slightly less at 12.1% (8.1,16.1).

Conclusions: The 20% fall in all cause mortality rates of women with diabetes mentioned on the death certificate in England and Wales over the 20 year period studied most likely reflects improved survival, whereas no significant change was found in men over this time. Overall rates of IHD deaths in women with diabetes mentioned on the death certificate have fallen since 1975-6. Rates of death caused by IHD in diabetic men have risen over the 20 year period, although it appears that the increase has plateaued since 1985-6 and that rates may be falling slightly. The major cause of death in patients with type 2 diabetes continues to be cardiovascular disease. Taking into account the limitations of death certificate data, these changes in IHD mortality in people with diabetes compare unfavourably with mortality trends in the general population, where there has been an approximately 30% overall reduction in IHD deaths over the 20 year period studied. Further initiatives to prevent cardiovascular disease in diabetic patients are therefore needed.

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2-HOUR GLUCOSE IS AN INDEPENDENT AND CONTINUOUS RISK FACTOR FOR MORTALITY FROM VARIOUS CAUSES

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Background and Aims: To assess whether 2-hour or fasting glucose is a continuous risk factor for mortality from all-cause and from cardiovascular disease (CVD).

Materials and Methods: Collaborative, prospective study of 20 European cohorts with baseline measurements of glucose and other risk factors. 25,038 subjects aged 30-89 years and not previously known as diabetic were followed up for 10 years on average with a total 306,774 person years accumulated.

Results: There were 4,315 deaths. Multivariate Cox proportional hazards analysis showed that inclusion of fasting glucose to the model did not add significant information over and above that of 2-hour glucose alone ($p > 0.10$ for various causes). The addition of 2-hour glucose to the model based on fasting glucose significantly improved the prediction ($p < 0.001$ for various causes). Hazards ratio adjusted for age, gender, cohorts and other CVD risk factors, corresponding to a 1% increase in fasting glucose concentration, was 1.92 (95%CI 1.52-2.42), 1.96 (1.34-2.87) and 1.87 (1.38-2.52), respectively, for deaths from all-causes, CVD and non-CVD. For 2-hour glucose, they were 1.57 (1.43-1.73), 1.64 (1.40-1.93) and 1.52 (1.35-1.71), respectively. When further adjusting for 2-hour glucose, the hazards ratio of fasting glucose reduced and became non-significant, i.e. 1.18 (0.91-1.53) for all-causes, 1.13 (0.73-1.74) for CVD and 1.20 (0.86-1.67) for non-CVD mortality. In contrast, the hazards ratios of 2-hour glucose did not change significantly after adjusting for fasting glucose, being 1.52 (1.37-1.69), 1.60 (1.33-1.93) and 1.47 (1.29-1.68), respectively.

Conclusions: The association between fasting glucose and mortality largely depended on the concomitant 2-hour glucose levels. 2-hour glucose concentration, but not fasting glucose, was an independent and continuous risk predictor for mortality.

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A SIMULATION MODEL FOR DIABETES MELLITUS TYPE I AND II: MODEL DESCRIPTION AND INTERNAL VALIDATION PROCESS WITH UKPDS, DCCT, AND WESDR DATA

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Background: A simulation model for IDDM and NIDDM was developed focusing on epidemiology and progression following various treatment regimes (TR). Because of the lack of validated models with real world data, this model was developed to provide a tool to gain more insight in the development of diabetes dependent from treatment regime and diabetic status. **Materials and Methods:** The model was designed in 3 steps. 1st the structure was developed independent of the availability of data. 2nd a systematic retrieval and review of the data was performed and, 3rd a model was constructed incorporating the data. Data from the DCCT, UKPDS, WESDR, HOPE studies, among others, were used for the input into the model. The model is a micro-simulation of a virtual patient cohort over n years. The following parameters have to be defined for running a simulation: Age, sex, age at diagnosis of diabetes, years since diagnosis, state of diabetes (hypoglycemic events (HYPO), albumin excretion rate, retinopathy (RP), cardiovascular status, smoking and living habits), and the current HbA1c level. TRs are divided into 2 major groups with intensive and conventional glycaemic control diabetes specific medications with insulin agents, oral antidiabetics and antihypertensives. Accompanying strategies like participation in diabetes teaching programs as well as screening programs for nephropathy and RP are also taken into account. Sequelae forecast by the model are HYPOs, microalbuminuria and the likelihood of progression towards ESRD, cardio- and cerebrovascular diseases, RP, blindness, clinically confirmed neuropathy, and lower extremity amputation. **Results:** The internal validation showed that the model correctly simulates the patient populations according to their underlying sources. E.g. for DCCT, UKPDS, and WESDR patient populations the difference between the original data and the model results lies between 1% and 24% relatively. For 30 validation runs the relative variation coefficient was between 0.93 and 4.78%, depending on the validated parameter. The simulation of 1000 patients over 10 years requires only 1 second. **Conclusion:** The developed model successfully passed the internal validation and appears to be robust and more reliable in comparison to previous developed models. In a next step, an external database not used for the development, should be employed as means for external validation.

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The Status of Diabetes Control in Asia - A Cross-sectional Survey of 24,317 Patients with Diabetes Mellitus in 1998.

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Background and Aims:

To establish the status of diabetes control in Asia, the DiabCare-Asia 1998 study collected data from 230 diabetic centers in Bangladesh, China, India, Indonesia, Malaysia, Philippines, Singapore, South Korea, Sri Lanka, Taiwan and Vietnam from March to December 1998.

Materials and Methods:

In this study, data were obtained either by patient interview during enrollment visit or by reviewing medical records for the most recent laboratory assessment and clinical examinations. Apart from basic patient data, information on the results of the most recent laboratory assessments and clinical examinations, various aspect of current diabetes management including complications, treatment, self-care and diabetes education were obtained from 24,317 patients. Blood samples were also collected during patients' visit for central assessments of HbA1c (normal range 4.7 to 6.4%).

Results:

The average centrally measured HbA1c was $8.6 \pm 2.0\%$ for 18,211 patients (82% of the analysis population). Mean HbA1c of the diabetic population in Malaysia, Indonesia, Korea, Bangladesh and Taiwan were significantly lower (all $p = 0.001$ except Malaysia $p = 0.0007$), while that of Philippines, Vietnam, China and India were all significantly higher (all $p = 0.0001$), than the grand mean. Of the patients with central HbA1c measurements, majority (55%) had values exceeding 8%, indicative of poor glycaemic control.

Conclusions:

Therefore, the single most important message of DiabCare-Asia 1998 would be that more than half of the Asian diabetes patients treated at diabetic centers, as represented by our study population, are not well controlled. Further therapeutic actions to improve glycaemic control are required to prevent chronic diabetic complications.

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THE EFFECT OF CASE MIX ON DIABETES CONTROL AND OUTCOMES IN TYPE 2 DIABETES

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Background and Aims: To quantify the way in which case mix effects diabetes control and outcomes using routinely collected electronic patient data. **Methods:** 674 958 annual patient summaries were amalgamated from 58 district diabetes registers. To ensure validity of data, records with a valid year of entry onto database, date of diagnosis, treatment type, an annual foot and eye summary, BP or BMI, biochemistry data, and no evidence of an absence of a macrovascular check were included, yielding 186 303 records. Of these 160312 could be identified as type 2 through date of diagnosis and treatment. Patients were designated an ethnicity group as Black, Indian or White, and assigned a Carstairs deprivation score based on postcode. The duration of diabetes from diagnosis by ethnic group and the existence of microvascular and macrovascular complication was assigned to each record. The mean duration of diabetes, glucose and lipid levels and rates of microvascular and macrovascular complication by ethnic category and deprivation quintile were investigated. **Results:** The mean duration (years) by quintile(Q) are [Q1(least deprived) 8.89, Q2-8.45, Q3-8.12, Q4-7.97 Q5-7.56]. The reduction in mean duration between each quintile was significant ($p < 0.003$). Black patients have significantly higher mean duration(8.07) compared to white (7.76) and Indian (8.07) ($p < 0.001$). HbA1c (%) significantly improves with increasing deprivation quintile ($p < 0.001$) [Q1-7.98, Q2-7.86, Q3-7.89, Q4-7.82, Q5-7.64] except Q2-Q3 ($p = 0.351$). Microvascular outcome rates significantly decrease with increasing deprivation quintile ($p < 0.001$) [Q1-56%, Q2-55%, Q3-53%, Q4-48%, Q5-46%] except Q1-Q2 ($p = 0.69$). Black patients have significantly higher rates of microvascular complication (53%) than white (49%) and Indian (32%) ($p < 0.003$). Mean total cholesterol levels (mmol/l) significantly increase with increasing deprivation quintile [Q1-5.14, Q2-5.12, Q3-5.27, Q4-5.35, Q5-5.36] ($p < 0.001$) with the exception of Q1-Q2 ($p = 0.21$). Macrovascular outcomes are significantly worse with increasing deprivation [Q1-24%, Q2-26%, Q3-28%, Q4-29%, Q5-30%] ($p < 0.002$) with the exception of Q4-Q5 ($p = 0.326$). **Conclusions:** There is a significant correlation between increasing deprivation and lower mean duration of type 2 diabetes. There is a similar correlation between increased deprivation, poorer lipid control and increased macrovascular complication. There is a significant correlation between increased deprivation, better glycaemic control and lower levels of microvascular complication. This apparent reduction may result from the lower mean duration in more deprived patients. Ethnicity appears to effect complication rates independently from deprivation.

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Epidemiology – Type 2 Diabetes: Mechanisms

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C-REACTIVE PROTEIN AND DIABETES MELLITUS TYPE 2

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Background and Aims: Low-grade systemic inflammation, marked by C-reactive protein (CRP), may cause insulin resistance. Higher CRP levels have been observed in patients with type 2 diabetes. The aims of the present study were to study the cross-sectional association between CRP and glucose metabolism and to study the predictive value of CRP for incident diabetes.

Materials and Methods: The Hoorn Study is a study of glucose tolerance among 2484 men and women, aged 50 to 74, which started in 1989. An extensive physical examination was performed in an age-, sex- and glucose-stratified random sample ($n = 631$). Diabetic status was assessed by two oral glucose tolerance tests at baseline and after 6 years of follow-up. Baseline CRP was assessed using a high-sensitivity assay.

Results: At baseline, the median for CRP in the NGT group was 1.3 mg/L. In subjects with IFG, IGT or both the median for CRP was 0.8, 2.2 and 2.5 mg/L respectively. The newly diagnosed patients had a median CRP of 2.3 compared to 2.7 mg/L in patients already known with diabetes. The OR for developing diabetes in 6 years was 2.7 (95% CI 1.2-6.5) in persons with high CRP compared to low CRP (cut off 85% percentile = 5.5 mg/L) after correction for sex and age. After adjustment for smoking and BMI the OR was 3.0 (95% CI 1.2-7.3). After further adjustment for fasting and postload glucose levels, the OR was reduced to 2.0 (95% CI 0.7-6.0).

Conclusions: CRP levels showed a cross-sectional association with glucose tolerance status, and in non-diabetic subjects CRP predicted the 6-year incidence of type 2 diabetes. Correction for glucose levels reduced the OR substantially, which may indicate that glucose is a part of the causal pathway of CRP involvement in diabetes aetiology.

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Impaired glucose tolerance in adults is associated with elevated serum levels of interleukin 6 but not of tumour necrosis factor alpha

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Background and Aims: Type 2 diabetes has been proposed to be associated with abnormal levels of products of innate immune cells. We studied whether such abnormalities might occur already in individuals at risk of type 2 diabetes, i.e. in adults with impaired glucose tolerance (IGT).

Materials and Methods: In the context of the Survey 2000 of the adult population in the Augsburg region (KORA) probands 55-74 years of age were invited to an interview, blood tests and an oral glucose tolerance test, if non diabetic. Sera were analysed for concentrations of IL-6, TNFalpha, IL-6 receptor, TNFalphaR1 and R2 by Sandwich ELISA.

Results: Mean levels of serum IL-6 were significantly higher in individuals with IGT compared to healthy controls [1.7 pg/ml (range <0.6-5.6) versus 0.9 pg/ml (range <0.6-2.3), $n = 40$ per group, $p < 0.002$]. Mean levels of IL-6 were also significantly elevated in individuals with type 2 diabetes [2.9 pg/ml (range <0.6-22.6), $n = 80$]. Levels of circulating IL-6 receptors in serum were not different between the three groups, suggesting an elevated level of bioactive IL-6 in IGT, similar to levels in type 2 diabetes. By contrast, mean serum levels of TNFalpha or of its receptors did not differ between the cohorts analysed.

Conclusions: These findings suggest that abnormally high serum levels of IL-6 occur in individuals at increased risk of type 2 diabetes. Immune mediators may possibly contribute to the pathogenesis of type 2 diabetes.

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SEASONAL VARIATIONS OF INSULIN SENSITIVITY, PLASMA GLUCOSE AND INSULIN SECRETION, VERSUS TEMPERATURE AND SUNSHINE.

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Background and Aims: Seasonal variations of HbA1c in diabetic subjects and of plasma glucose (PG) in healthy subjects have been reported but it is not known if these are due to seasonal variations in insulin sensitivity. The aim was to reveal any seasonal variations of insulin sensitivity, PG and insulin secretion in a population-based cohort of 70-year old men (n=1221), and if such variations were related to surrounding temperature (ST) and hours of sunshine (HS).

Materials and Methods: The subjects were investigated between January 1991 and December 1995. The investigations, randomly carried out throughout the year, were a hyperinsulinemic euglycaemic clamp, measurements of PG and insulin from an oral glucose tolerance test (OGTT). In regression analyses, indicator variables for summer/winter season (May-September/October-April; 0/1) and for month (January-December, labelled 1-12) whereas non-linear relationships were tested with month and month squared, ST and HS were analysed as explanatory variables. **Results:** During the winter, insulin sensitivity index [M/I (100.mg/kg bw/min/mU/l)] was lower (4.8 vs 5.3, p<0.001), AUC for insulin during the OGTT was higher (1190 vs 1040 mU/l, p=0.001) and fasting PG was slightly higher (5.8 vs 5.7 mmol/l, p=0.1). There was a significant association between ST and M/I (p<0.002) but not between HS and M/I (p<0.31). When seasonal variation of M/I was further adjusted for ST, the effect of season on M/I became non-significant (p=0.51). When month and month squared combined were regressed on M/I, both were significantly related to M/I (p=0.005 and p=0.03) but when adjusted for ST both became non-significant. **Conclusion:** Seasonal variation in insulin sensitivity induced only a small variation in plasma glucose and insulin secretion compensates for the variation of insulin sensitivity. Seasonal variation of insulin sensitivity was associated with the surrounding temperature. The results may have implications on glycaemic control in diabetic subjects and for the design of clinical trials with insulin sensitivity as an end point.

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Insulin resistance and beta-cell dysfunction as predictors of incident glucose intolerance and type 2 diabetes, the Hoorn Study.
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Background and Aims: The variability of the Oral Glucose Tolerance Test (OGTT) derived fasting and 2-hour glucose values is high. Guidelines for clinical practice require duplicate (i.e. confirmed) abnormal fasting or post-load values to categorise persons as having diabetes. The aims of the present study were to determine the incidence of diabetes and to identify predictors for the changes in glucose tolerance, as assessed with duplicate OGTTs, in the general population.

Material and methods: In 1989-90, 2484 men and women aged 50-75 years, completed an OGTT. An age, sex, and glucose tolerance stratified random sample of 631 subjects had a second OGTT. Of the non-diabetic subjects at baseline a total of 250 had again a duplicate OGTT after a mean follow-up of 6 years. Subjects with confirmed normal glucose tolerance (NGT) were categorised as NGT. Subjects with one or two times glucose values in the impaired fasting glucose or impaired glucose tolerance (IGT) range were categorised as impaired glucose metabolism (IGM). Subjects with two times an OGTT in the diabetes range (WHO98) were categorised as type 2 diabetes.

Results: Of the subjects categorised as NGT as baseline (n=120) only one person developed type 2 diabetes at follow-up. Of the subjects with IGM (n=130), 38 (29.2%) developed type 2 diabetes. Of those with newly detected type 2 diabetes (n=67) at baseline, 40 subjects had no medical treatment at follow-up and had a duplicate OGTT. Of these, 6 subjects returned to IGM (15%). Fasting plasma glucose and waist/hip ratio in NGT were significantly (p<0.05) related to the development of IGM while fasting plasma glucose and 2-h post-load plasma specific insulin were significantly (p<0.05) related to the development of type 2 diabetes, while only fasting pro-insulin levels predicted conversion from IGT to type 2 diabetes.

Conclusion: These results with duplicate OGTTs at baseline and follow-up indicate that subjects with confirmed NGT have a very low risk of developing type 2 diabetes. This study confirms the previously reported sequence of events, i.e. that insulin resistance, as reflected by high 2-hour specific insulin levels is the major determinant of IGM, while impaired beta-cell function, as reflected by high fasting pro-insulin levels, is a determinant of the occurrence of type 2 diabetes.

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A HIGH WHITE BLOOD CELL COUNT PREDICTS WORSENING OF INSULIN SENSITIVITY AND DEVELOPMENT OF TYPE 2 DIABETES IN PIMA INDIANS

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Background and Aim: It has been proposed that chronic inflammatory processes and/or infections may contribute to the development of type 2 diabetes. Markers of inflammation were found to predict diabetes in mixed Caucasian and African-American population. The aim of the present study was to examine whether a high white blood cell count (WBC), a marker of inflammation, predicts worsening of insulin sensitivity and/or insulin secretion and the development of type 2 diabetes in Pima Indians. **Materials and Methods:** We measured WBC in 260 normal glucose tolerant Pima Indians [180M/80F, age 27±6y, body fat 31±8%, WBC 7849±1717 cells/mm³ (mean±SD)] who were characterized for body composition (hydrodensitometry or DXA), glucose tolerance (75g OGTT), fasting plasma insulin concentrations (INS), insulin sensitivity [M, hyperinsulinemic clamp] and acute insulin secretory response to glucose (AIR, 25g intravenous glucose challenge). Forty-eight subjects developed diabetes over an average follow-up of 5.0±4.2 y. In 73 subjects who remained non-diabetic, follow-up measurements of M and AIR were available. **Results:** At baseline, WBC was related to body fat (r=0.36; p=0.0001), INS (r=0.29; p=0.0001), and M (r=-0.24; p=0.0001). In a proportional hazard analysis with adjustment for age and sex, a high WBC predicted diabetes (relative hazard 90th vs. 10th centile (95%CI): 5.3 (2.2-12.8); p=0.0002). The predictive effect of WBC persisted after additional adjustment for body fat, M and AIR (relative hazard 3.7 (1.4-9.8); p=0.003). A high WBC at baseline predicted a decrease in M (p=0.01), but not in AIR, after adjustment for sex, baseline age, change in body fat and follow-up duration. **Conclusion:** We conclude that in Pima Indians, an elevated WBC predicts type 2 diabetes. This effect is, in part, mediated by a worsening of insulin sensitivity. Our findings are consistent with the hypothesis that chronic inflammatory processes may play a role in pathogenesis of type 2 diabetes.

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BODY COMPOSITION AND INCIDENT DIABETES IN JAMAICAN ADULTS

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Background and Aims: Anthropometric indices of obesity such as the body mass index (BMI) and central adiposity such as waist circumference, waist to hip ratio (WHR) and waist to height ratio (WHTR) have been associated with diabetes and various cut-off points have been proposed to indicate thresholds for intervention. There are few data on the performance of these indices in predicting incident diabetes in the developing world where the burden of obesity and diabetes is high.

Materials and Methods: A cohort of 724 non-diabetic adults (287 men and 437 women), aged 25-74 y and resident in Spanish Town, Jamaica were followed for a mean of 4 years. At baseline, height, weight, waist and hip circumferences were measured using standardised protocols. Participants had fasting and 2-hour post challenge glucose concentrations measured at baseline and follow-up. The diagnosis of incident diabetes was based on 1999 WHO criteria. **Results:** Mean and standard deviation (SD) BMI in men and women at baseline were 23.5 (4.2) and 27.7 (6.5) kg/m², respectively. Mean (SD) waist circumference was 80.3 (11.7) cm in men and 82.6 (12.6) cm in women. There were 51 cases of incident diabetes (17 men and 34 women). BMI, waist circumference, WHR and WHTR were investigated in separate Cox proportional hazards regression models including age and sex. All indices were independent predictors of diabetes and regression models had similar likelihood ratio chi-square values indicating that none of these indices were clearly superior. Receiver operating characteristics curves (ROC) were used to determine optimal cut-off points for these indices. The area under the ROC curve (95% confidence interval) for BMI was 0.74 (0.59-0.88) for men and 0.62 (0.51-0.72) for women. For waist circumference these values were 0.78 (0.65-0.91) in men and 0.61 (0.50-0.71) in women. Similar results were obtained for WHR and WHTR. Optimal cut-off points for BMI were 24.8 kg/m² (men) and 29.3 kg/m² (women). For waist circumference these were 88 cm and 84.5 cm for men and women, respectively. Corresponding values for WHR were 0.87 and 0.80 and for WHTR, 0.51 and 0.54.

Conclusions: BMI, waist circumference, WHR and WHTR all predicted incident diabetes and no index was clearly superior to the others. Cut-off points for waist circumference and WHR were similar to those proposed in developed countries. Of the indices examined, waist circumference is easiest to interpret and could be useful in health promotion as an alternative to BMI.

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SKINFOLD THICKNESS MEASUREMENTS DISTINGUISH BETWEEN TRANSIENT AND PERSISTENT GLUCOSE INTOLERANCE.

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Background and Aims: Impaired glucose tolerance is an unstable state. With repeated testing many revert to normal, other remain IGT. Subjects with persistent IGT have the greatest risk of developing type 2 diabetes mellitus. The aim of the present study was to evaluate whether adding anthropometric measurements to glucose tolerance testing could improve the prediction of persistent IGT. **Materials and methods:** 175 subjects with glucose intolerance (IGT and newly diagnosed type 2 DM) were invited for a second OGTT. When revert to normal subjects were classified as having transient glucose intolerance (tGI), in all other cases as having persistent glucose intolerance (pGI). Anthropometric measurements were performed during the second test. Body fat% was calculated from the sum of skinfolds according to the method of Durnin and Womersley.

Results: tGI was diagnosed in 82 subjects (47%); pGI in 93 (53%). pGI was associated with lower fasting and 2-hour glucose levels at the initial test. Furthermore pGI was associated with higher BMI (29.9 ± 0.4 vs. 28.0 ± 0.4 kg/m²; $P < 0.001$), elevated waist (103.3 ± 1.0 vs. 98.3 ± 1.1 cm; $P < 0.01$) elevated WHR (0.98 ± 0.01 vs. 0.95 ± 0.01 ; $P < 0.05$) and increased percentage body fat (men: 33.9 ± 0.5 vs. 31.1 ± 0.8 %; $P < 0.01$; women 43.5 ± 0.4 vs. 42.0 ± 0.5 %; $P < 0.05$). Skinfolds were higher in pGI compared to tGI. After correction for age, sex and family history of DM logistic regression indicated that glucose levels during the initial test most strongly predict persistent IGT ($P < 0.001$), followed by BMI, body fat%, skinfolds and waist. WHR did not distinguish between tGI and pGI ($P=0.07$). When also adjusted for glucose levels during the initial test only body fat% and subscapular skinfold thickness distinguished between transient and persistent glucose intolerance ($P < 0.01$).

Conclusions: Percentage body fat and subscapular skinfold thickness, distinguish between transient and persistent glucose intolerance, suggesting that subcutaneous adipose tissue, especially centrally located, may have additional predictive value of determining persistent glucose intolerance.

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DIETARY PATTERNS AND RISK OF TYPE 2 DIABETES MELLITUS IN U.S. MEN

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Background and aims: The role of diet in the development of type 2 diabetes remains unsettled. Therefore, we examined the association between major dietary patterns and risk of type 2 diabetes mellitus. **Materials and methods:** We prospectively followed 42,626 U.S. male health professionals, aged 40 to 75 years and free of diagnosed diabetes, cardiovascular disease and cancer in 1986. Using factor analysis based on data from food frequency questionnaires, we identified and validated two major dietary patterns that we labeled 'prudent' (characterized by higher consumption of vegetables, fruit, fish, poultry and whole grains) and 'Western' (characterized by higher consumption of red meat, processed meat, French fries, high fat dairy, refined grains, and sweets and desserts). During 12 years of follow-up, we ascertained 1327 incident cases of type 2 diabetes. Relative risks and 95% CIs were adjusted for potential confounders, including BMI, physical activity and cigarette smoking. **Results:** The prudent dietary pattern score was associated with a modestly lower risk of type 2 diabetes (relative risk for extreme quintiles, 0.85 [CI 0.71-1.01]). In contrast, the Western dietary pattern score was associated with an increased risk of type 2 diabetes (relative risk, 1.54; CI 1.27-1.86; P trend < 0.0001). The combination of a high Western dietary pattern score (highest quintile) and obesity ($BMI \geq 30$ kg/m²) was associated with a particularly high risk of type 2 diabetes (relative risk 10.9; CI 7.84-15.2 relative to lowest quintile and $BMI < 25$ kg/m²). **Conclusion:** Our findings suggest that a Western dietary pattern is associated with a substantial increase in risk of type 2 diabetes.

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Beta Cell Growth and Differentiation

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INCREASED BETA-CELL PROLIFERATION AFTER 70% PANCREATECTOMY IN MICE OVEREXPRESSING SHB AND GTK IN BETA-CELLS

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Background and Aims: GTK (BSK1/YK), a tyrosine kinase of the SRC-family, regulates cellular processes such as growth, differentiation and survival. A tyrosine residue in the C-terminal tail, Y504, is a negative regulator of GTK kinase activity. SHB, a SH2 domain adapter protein, is phosphorylated in response to GTK-overexpression in PC12 cells and RINm5F cells. SHB- and GTK-transgenic mice have a larger beta-cell mass and exhibit an increased cytokine-induced beta-cell death. The aim of the present study was to assess beta-cell growth in response to partial pancreatectomy and glucose tolerance in transgenic mice expressing Y504F-mutated GTK or SHB under the control of the rat insulin promoter. **Methods:** A 70% pancreatectomy (Px) or sham pancreatectomy was performed on GTK-transgenic, SHB-transgenic or control mice (4-5 month of age). On day 5 after the operation the mice were injected with [³H]-thymidine and the beta-cell labelling index was assessed. 3-month-old GTK-transgenic and control mice were fasted overnight followed by an intravenous glucose injection (250 ml of a 30% solution) and blood glucose was determined at 0, 10, 30, 60 and 120 min. Insulin was assessed from blood samples collected at 0, 10 and 30 min (Mecodia Ultrasensitive Rat Insulin ELISA).

Results: There were no significant differences in beta-cell proliferation between the sham-operated mice (control: $0.7 \pm 0.2\%$, GTK: $0.7 \pm 0.4\%$, SHB: $1.5 \pm 0.6\%$). 70% Px stimulated beta-cell regeneration in all groups but there was a significantly increased beta-cell thymidine incorporation after Px in the SHB-transgenic ($3.8 \pm 1\%$) and GTK-transgenic ($5.3 \pm 1.2\%$) mice compared to the control mice ($1.7 \pm 0.4\%$). There was no difference in the glucose tolerance between control and transgenic mice that had been housed in cages with two or more animals. However, three-month-old male transgenic mice that had been separated from each other from three weeks of age exhibited a slower glucose disappearance rate compared to single-caged control mice. Moreover the transgenic mice showed elevated basal levels of insulin but failed to further increase the insulin secretion in response to the glucose injection.

Conclusion: The results suggest that GTK and SHB have beneficial effects on beta-cell proliferation when stimulated with partial pancreatectomy, thus partly explaining the increased beta-cell area previously observed in these mice. However, GTK-overexpression in beta-cells also seems to affect insulin secretion, resulting in impaired glucose tolerance under certain conditions.

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STIMULATION OF ISLET GROWTH AND AMELIORATION OF DIABETES BY COMBINATION THERAPY WITH TGF-ALPHA AND GASTRIN.

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Background and Aims: The growth factors, TGF α and gastrin, have been reported to affect growth and differentiation of pancreatic islet B-cells.

Materials and Methods: The effects of systemic administration of TGF- α /gastrin on islet cell growth and glucose tolerance were examined in non-diabetic, streptozotocin (STZ)-induced diabetic and diabetic Zucker rats. All study animals were treated (daily i.p. injection) with a combination of TGF- α (4ug/kg BW) and gastrin (4ug/kg BW), or vehicle (PBS) alone, for 10 days. Pancreatic immuno-histochemistry was used to quantitate B-cell neogenesis (number of single B-cell foci expressed as a % of total B-cells) in lean and obese diabetic Zucker rats.

Results: No TGF- α /gastrin treated rats showed any changes in body weight or fasting blood glucose levels compared to vehicle (PBS)-treated controls. TGF- α /gastrin treatment increased pancreatic insulin content, compared to PBS controls, in non-diabetic (60.6 ± 8.7 vs 20.6 ± 6.0 ug insulin/mg protein; $p < 0.05$) and STZ-diabetic (26.7 ± 8.9 vs 6.1 ± 2.1 ug insulin/mg protein; $p < 0.05$) rats. Glucose tolerance (IPGTT - 2g glucose/kg BW) was normalised in STZ-diabetic rats treated with TGF- α /gastrin, with blood glucose returning to fasting levels within 2 hours of receiving a glucose load. TGF- α /gastrin increased B-cell neogenesis, compared to controls, in both lean (10.5 ± 0.9 vs 3.9 ± 1.1 %; $p < 0.004$) and obese (11.2 ± 1.0 vs 4.2 ± 1.1 %; $p < 0.002$) Zucker rats.

Conclusions: Thus TGF- α /gastrin treatment appears to increase islet B-cell growth and improve glycaemic control in diabetic animal models, suggesting a possible therapeutic role in preventing the progression from impaired glucose tolerance to overt disease in type II diabetes.

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DIFFERENT MAP-KINASE PATHWAYS INVOLVED IN ISLET CELL DIFFERENTIATION INDUCED BY BETACELLULIN AND NEUREGULIN-4

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Background and Aims: We have previously shown that islet morphogenesis is impaired and beta-cell differentiation delayed in mice lacking functional EGF receptors (EGFR^{-/-}). We have also demonstrated that the EGF-R ligand betacellulin (BTC) and the erbB-4 ligand neuregulin-4 (NRG-4) direct the differentiation of developing mouse islet cells towards beta- and delta-cells, respectively. The present study aims to clarify the intracellular signalling pathways activated by BTC and NRG-4.

Materials and Methods: Collagenase digested normal embryonic (E16) mouse pancreatic tissue was cultured for 48 h, serum-starved and stimulated for 10 min with 100 ng/ml of either BTC, NRG-4 or EGF. Tissues were then lysed and resolved by SDS-PAGE electrophoresis before transfer onto nitrocellulose. Tyrosine phosphorylation of the MAP-kinases Erk1/2, Akt, p38 and Jnk, and the p53 subunit of PI3 kinase was studied by immunoblotting. The results are based on three independent experiments.

Results: BTC, NRG-4 and EGF stimulated PI3Kp85 phosphorylation similarly (1.7 to 2.3-fold). While none of the factors significantly affected the phosphorylation of either Akt, JNK or p38, Erk1/2 was potently activated by EGF (16-fold increase in phosphorylation) and BTC (6-fold), but not by NRG-4.

Conclusions: BTC-induced beta-cell differentiation involves the Erk1/2 pathway, whereas NRG-4 induced delta-cell differentiation does not. Furthermore, though both EGF and BTC activated Erk1/2, only BTC stimulated beta-cell differentiation. Thus, yet unidentified signalling pathways are involved in mediating BTC action.

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Hepatocyte Growth factor (HGF) induces differentiation of pancreatic ductal cells into insulin-producing cells

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Background and Aims: HGF and its receptor c-met have been shown to be involved in development, growth and regeneration of different organs and tissues. Data from in vitro and in vivo studies suggest that the HGF-cmet system may play a role in promoting beta cell neogenesis. The aim of the study was to investigate whether pancreatic ductal epithelial cells could be induced to differentiate into insulin-producing cells by exposing them to hepatocyte growth factor (HGF).

Materials and Methods: Rat pancreatic ductal cells (ARIP) were first examined by immunohistochemistry for the expression of insulin and the HGF receptor, c-met. The expression of c-met was also evaluated by western blot analysis. In addition, RT-PCR was performed to detect pancreatic-duodenal homeobox-1 (PDX-1) mRNA. ARIP cells were cultured at increasing concentrations of HGF and analyzed at different time points for the expression of insulin by immunohistochemistry. In addition, insulin released into the medium was analysed by radioimmunoassay (RIA).

Results: A positive immunoreactivity for insulin was detected in ARIP cells cultured in the presence of HGF (50 ng/ml) for 48 hours; in contrast ARIP cells cultured with vehicle alone were insulin negative. A positive immunoreactivity for c-met was detected in ARIP cells treated with vehicle alone. The constitutive expression of c-met receptor was also demonstrated by western analysis. RT-PCR analysis showed that PDX-1 gene is constitutively transcribed in ARIP cells. When cultured in the presence of HGF, ARIP cells exhibited a dose-dependent response of insulin secretion with a plateau of the response with 50 ng/ml of HGF. The analysis of the time course of the insulin secretory response revealed a maximal secretion at 48 hours and a plateau at 72 hours followed by an early decline at later time points.

Conclusions: We provide further evidence to support the observation that pancreatic ductal cells in vitro could be trans-differentiated into insulin secreting-cells by means of culturing with HGF.

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OVEREXPRESSION OF FGF10 IN THE DEVELOPING MOUSE PANCREAS PERTURBS ENDOCRINE CELL DIFFERENTIATION.

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Background and Aims: FGF-signalling plays a key role in the development of the mouse embryo and in particular the development of organs which derive from epithelial-mesenchymal interactions. We have previously shown that key components of the FGF family are expressed in the adult mouse pancreas and that impairment of signalling via FGFR1c leads to diabetes as a result of b-cell dysfunction. Our aim was to investigate the role that signalling via FGFR2b may play in the developing mouse pancreas, by overexpressing FGF10.

Materials and Methods: The transgenic construct was made by inserting a 4.5-kilobase (kb) NotI-NaeI fragment located immediately upstream of the *Ipf1/Pdx1* gene into a vector carrying a polyA site and a 750bp NotI/NcoI mouse FGF10 cDNA (*Ipf1/fgf10*). We generated transgenic mice by pronuclear injection of the purified (NotI-BamHI) (1.8 ng/ml) into F2 B6/CBA hybrid oocytes as described. The genotype was determined by PCR analysis of genomic DNA extracted from tail biopsies. Immunohistochemistry and in situ analysis was performed at various stages of pancreas development.

Results: *Ipf1/fgf10* mice were born alive where approximately 50% of all transgenic offspring presented with severe hyperglycaemia (blood glucose levels were 18.4 ± 1.2 and 4.2 ± 0.7 mM for *Ipf1/fgf10* and wild type littermates respectively). The remaining animals appeared healthy with fed blood glucose levels within the normal range. However, upon examination of the pancreas, derived from both hyperglycaemic and normoglycaemic animals, (e13, 15, 17, P1 and 7 weeks) the gross morphology revealed an enlarged and condensed pancreas. Immunohistochemical analysis using a battery of pancreatic markers revealed ductal hyperplasia in the transgenic pancreas, which was most apparent from e15 onwards. The animals presenting with hyperglycaemia, had few differentiated *glut*⁺ and *ins*⁺ cells, which may explain their elevated blood glucose levels. In addition female adult *Ipf1/fgf10* animals had a fluid-filled cyst in the dorsal pancreas position. This cyst had a thin layer of tissue which, was *Ipf1*⁺, *p48*⁺ and carboxypeptidase A positive, but very few insulin positive cells were detected. Both male and female derived ventral pancreas had a condensed morphology and appeared to harbour more insulin and glucagon producing cells as compared to the dorsal pancreas. However, very few Islet structures were observed in both dorsal and ventral derived pancreas, instead the cells were in a scattered arrangement.

Conclusions: Overexpression of FGF10 in the developing mouse pancreas leads to ductal hyperplasia and perturbed endocrine cell differentiation.

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Restoration of glucose-responsive insulin secretion following formation of cell clusters in the poorly responsive betaTC-3 cell line.

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Background and Aims: Investigation of molecular aspects of insulin secretion is restricted by poor availability of human and animal islets of Langerhans. Insulin-secreting beta-cell lines have been developed, but these often show poor responses to glucose when analysed as monolayers or cell suspensions. Normal islets also show reduced secretory responses when dissociated into single cells. We investigated whether formation of islet-like clusters increased the ability of the poorly responsive betaTC-3 cell line to secrete insulin on stimulation with glucose or activators of the cAMP pathway (forskolin plus 3-isobutyl-1-methylxanthine (IBMX)) or of the protein kinase C pathway (phorbol 12-myristate 13-acetate (PMA)).

Materials and Methods: Beta TC-3 cells were grown as monolayers, or formed into clusters by culture on gelatin coated plates. Morphology of cells was investigated by light and electron microscopy. Monolayers or cell clusters (0.5 million cell equivalents) were perfused with sequential changes of medium from 0 mM glucose to 20 mM glucose, then to 20 mM glucose with either 10 uM forskolin & 100 mM IBMX, or 500nM PMA. Medium was returned to 0 mM glucose and cells re-perfused with 20 mM glucose. Perifusates were collected every 2 min and assayed for insulin secretion in a single radioimmunoassay with intra-assay CV of 5.2%. Insulin secretion was expressed as % basal secretion.

Results: When grown on gelatin, beta TC cells formed islet-like clusters, which were well granulated and demonstrated inter-cellular tight gap-junctions. Clusters showed increased insulin secretion in response to 20 mM glucose (peak 531±61% basal, p<0.01). Forskolin + IBMX (958±441, p<0.05) and PMA (735±116) compared to monolayers (202±46, 144±19, and 214±17, respectively). Prior stimulation enhanced the magnitude of subsequent stimulation (priming) in clusters, but not in monolayers.

Conclusions: The results demonstrate the importance of islet-like structure for appropriate insulin responses to secretagogues. Formation of cell clusters may have implications for function of engineered beta cells for transplantation.

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UP-REGULATION OF E-CADHERIN SIGNALLING ELEMENTS AND IMPROVED SECRETORY RESPONSES OF MIN6 PSEUDOISLETS

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Background and Aims: The MIN6 cell line forms three-dimensional aggregates (pseudoislets; PIs) when grown on gelatin. We have previously shown that PIs express more of the Ca^{2+} -dependent cell adhesion molecule, E-cadherin (ECAD) and that they show improved secretory responses over monolayer (M) cells to nutrient secretagogues. We have now investigated the time-course of PI formation, the change in expression of ECAD and α - and β -catenins in PIs, and the secretory responses of PIs to non-nutrient secretagogues. **Materials and Methods:** MIN6 PIs were formed in gelatin-coated tissue culture flasks. ECAD, α -catenin and β -catenin expression were detected by PAGE and Western blotting. Insulin secretion from perfused MIN6 cells and PIs was measured by radioimmunoassay. **Results:** MIN6 cells formed clusters that were visible as multi-cellular aggregates after 2-4 days in culture. By day 7 these aggregates were indistinguishable from primary islets by light microscopy. Western blotting of protein-matched M and PI extracts indicated that ECAD, β -catenin and α -catenin expression were all up-regulated in PIs harvested at day 7. ECAD expression was increased within 1 day, and this was maximal by day 4. The membrane-depolarising stimuli KCl (20mM) and tolbutamide (100 μ M) both caused rapid and sustained increases in insulin secretion from PI (328 \pm 84% and 423 \pm 138% basal, respectively, n=4) which were significantly ($P<0.05$) greater than those from M cells. Similar results were obtained using the PKC activator PMA (500nM) and the adenylate cyclase activator forskolin (10 μ M), both of which produced maintained elevations in secretion from PI, but had only transient effects in M cells. Carbachol (500 μ M) caused a sustained plateau increase in 20mM glucose-induced insulin secretion from PI, but only produced a short (6 min) peak in insulin secretion from M cells. **Conclusions:** These data suggest that the up-regulation of ECAD, α -catenin and β -catenin occurs over the same time-course as pseudoislet formation, and that β -cells within islet-like structures exhibit an overall enhancement of insulin secretion in response to divergent extracellular signals.

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REGULATION OF INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN-1 SECRETION IN THE GLUCOSE-SENSITIVE RAT PANCREATIC BETA CELL LINE INS-1

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Background and Aims: Insulin-like growth factor-binding protein-1 (IGFBP-1) regulates the activity of the IGFs and has IGF-independent actions on cell motility and apoptosis. We have previously characterised the regulation of rat IGFBP-1 in vivo and in vitro. In H4-II-E rat hepatoma cells, insulin is a potent inhibitor of IGFBP-1 secretion, glucocorticoids are stimulatory and glucose has no effect. We have recently identified AMP-activated protein kinase (AMPK) as a novel regulatory pathway for IGFBP-1, stimulating its secretion in H4-II-E cells. IGFBP-1 is known to be expressed in INS-1 cells, however little is known about its regulation in these cells. The aim of this study was to determine whether INS-1 cells also secrete IGFBP-1 and, if so, to compare the pattern of regulation in a pancreatic beta cell line to that of hepatic IGFBP-1.

Materials and Methods: Studies were performed in monolayer cultures of INS-1 cells. After a 6-h serum-free period, cells were exposed to effectors in the absence of serum for a further 18h. Conditioned medium was collected and IGFBP-1 measured by sensitive immunoassay and by immunoblotting after SDS-PAGE.

Results: IGFBP-1 was secreted by INS-1 cells. Immunoreactive IGFBP-1 was detected in the immunoassay and was also visualised by Western immunoblotting. Under these culture conditions dexamethasone, 100ng/ml, stimulated IGFBP-1 secretion 4-fold, and increasing the glucose concentration from 11 to 20 mM inhibited IGFBP-1 by approx 50%. Activation of AMPK using 5-aminoimidazole-4-carboxamide-riboside inhibited IGFBP-1 secretion up to 50%.

Conclusions: (i) The glucose-sensitive pancreatic beta cell line INS-1 secretes IGFBP-1; (ii) similar to hepatic IGFBP-1, glucocorticoids stimulate pancreatic IGFBP-1 secretion; and (iii) in contrast to liver, glucose inhibits IGFBP-1 secretion, and activation of AMPK is also inhibitory. We speculate that IGFBP-1 may play a role in INS-1 cell function, independently or by modulating local IGF activity.

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EXPRESSION OF ISLET MARKERS IN STEM CELLS.

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Background and aims: Stem cells have the ability to differentiate to any cellular lineage, after a differentiation process. Developmental studies have shown the involvement of some transcription factors in pancreas development. The aim of this study is to investigate the role of the above mentioned transcription factors in a mouse embryonic stem cell line (D3) and in an adult human intestinal stem cell line (HISC), in their process of differentiation to islet cells. **Materials and methods:** D3 and HISC have been differentiated following a standard differentiation process, consisting of embryoid bodies (EB) formation and their subsequent culture in monolayer. The visualization of the differential expression of the following genes, PDX-1, ngn-3, isl-1, NeuroD/Beta2, Nkx2.2, Nkx6.1, Pax4, Pax6, p48, insulin, glucagon, somatostatin, PP, glucokinase, GLUT-2 and SUR-1, has been done by RT-PCR and immunohistochemistry. **Results:** Undifferentiated D3 cells show markers related with pancreas development, such as ngn-3, Isl-1, NeuroD/Beta2, Nkx2.2, Pax4 and Pax6. Moreover, these undifferentiated cells also show markers from mature endocrine pancreatic cells, such as PP and SUR-1. On the other hand, markers like PDX-1 appear in late differentiation stages. Undifferentiated HISC cells show the same markers related with pancreas development, except Nkx2.2 and PDX-1. On the contrary, HISC cells are positive for glucokinase. **Conclusion:** These data suggest that undifferentiated D3 and HISC stem cells already express certain pancreatic markers. On the contrary, the presence of other markers, like PDX-1 and glucokinase, change in the different lines and along the differentiation process, suggesting that differentiation and maturation protocols can be used to guide these cells to a islet cell precursor population.

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Gene Expression in Beta-Cells

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DIFFERENTIAL GENE EXPRESSION IN GLUCOSE-RESPONSIVE AND NON-RESPONSIVE PANCREATIC β -CELL (MIN6) SUBLINES.

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Background and Aims: To date only a few candidate genes have been proposed to be involved in maintaining the differentiated state of a pancreatic β -cell. To identify genes involved in regulated insulin secretion, we have established and characterised two sublines derived from the insulin-secreting mouse pancreatic β -cell line MIN6, designated B1 and C3.

Materials and Methods: MIN6 cells were subcloned by limiting dilution. To study their insulin-secretory properties, cells were incubated for 1 h in basal conditions (2.8 mmol/L glucose) and subsequently for 1 h in stimulated conditions (except KCl = 10 min). Data are mean \pm SE. To identify genes differentially expressed in the two cell lines we have applied suppression subtractive hybridization (SSH).

Results: B1 and C3 cells are morphologically different but do not differ significantly in cellular insulin content (4.1 \pm 0.7 vs. 5.2 \pm 0.4 μ g/10⁶ cells). B1 responded to glucose in a concentration and confluence-dependant manner. At 16.7 mmol/L glucose, these cells at 75% confluence released 14.7 \pm 1.1% insulin content/h vs. 3.3 \pm 0.1% at 2.8 mmol/L glucose (p <0.01). C3 did not respond to glucose 2.8-16.7 mmol/L regardless of cell confluence. Fold-stimulation of insulin release by other secretagogues (at 2.8 mmol/L glucose) was (B1 vs. C3): 20 mmol/L leucine 4.6 \pm 0.2 vs. 0.8 \pm 0.1; 20 mmol/L arginine 1.4 \pm 0.2 vs. 0.7 \pm 0.1; 1 mmol/L IBMX 3.6 \pm 0.6 vs. 2.3 \pm 0.4; 100 nmol/L PMA 16.8 \pm 4.0 vs. 2.1 \pm 0.5; 30 mmol/L KCl 42.8 \pm 5.2 vs. 15.6 \pm 5.2. By SSH we have identified two potentially interesting genes, which are strongly under-expressed in the defective clone C3 as compared to B1. This was confirmed by Northern blot and immunofluorescence. They code for the neurofilament subunits NF-L and NF-M, which belong to the family of intermediate filament proteins. Such proteins are involved in cellular architecture and their lowered expression could be implicated in the cellular/secretory defects of C3.

Conclusions: Secretion studies show an impaired insulin secretion in the C3 cells in response to glucose and to the other tested secretagogues as compared to the B1 cells. These two sublines should be useful for identification of genes indispensable for normal regulated insulin secretion.

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HIGH GLYCAEMIC INDEX DIETS INDUCE CHANGES IN PANCREATIC INSULIN SECRETION AND GENE EXPRESSION

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Background and Aims: Prolonged intake of high glycaemic index (GI) diets increase the pancreatic insulin response to glucose both in vivo and in vitro in rodents. This has been implicated in the development of insulin resistance. The primary and most characterized pathway for glucose stimulated insulin release involves pancreatic glucose metabolism. The two major components in this pathway, GLUT-2 and glucokinase (GK) ('the glucose sensor') play crucial roles in the maintenance of normal glucose stimulated insulin secretion. The aim of this study was to determine the effect of high GI diets on GLUT-2 and GK expression.

Materials and Methods: Rats (n=12) were maintained on one of two diets (68% carbohydrate) for 3 weeks. Diets contained either low GI starch "amylose" (60% amylose 40% amylopectin) or the high GI carbohydrate "polycose" (100% polycose powder). Intravenous glucose tolerance tests (IVGTT) were performed (1g glucose/kg body wt) at 3 weeks. Expression of islet GLUT-2, GK and pre-proinsulin genes were measured by RT-PCR (expressed as a ratio to b-actin).

Results: Compared to amylose, polycose diet significantly increased both plasma glucose (AUC 25548 vs 155453 mmol.l-1.2h, p =0.02) and insulin (AUC 48 \pm 6 vs 2544.7mmol.l-1.2h, p =0.03) when given as a single meal. At 3 weeks there was no difference in glucose response to IVGTT between the two diets (AUC 320 \pm 48 vs 309 \pm 40mmol.l-1.h, p =0.9). Fasting insulin was the same in each group (225 \pm 105 vs 105 \pm 15pmol/l, p =0.14) however, insulin response to IVGTT was elevated in polycose rats at 2 minutes after glucose infusion (3900 \pm 675 vs 1350 \pm 90pmol/l, p =0.04). Expression of GLUT-2 was significantly reduced in the polycose group compared with amylose group (0.03 \pm 0.01 vs 0.3 \pm 0.09, p =0.02), as was GK expression (0.01 \pm 0.006 vs 0.2 \pm 0.05, p =0.006). Pre-proinsulin gene expression was not different between groups (0.64 \pm 0.09 vs 0.68 \pm 0.14, p =0.9).

Conclusions: This unexpected 90% decrease in the expression of the 'glucose sensor' suggests an adaptive response to chronic, intermittent post prandial hyperglycaemia and suggests an alternate pathway for the insulin hypersecretion that accompanies high GI diets.

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TRANSCRIPTIONAL REPRESSOR DREAM INFLUENCES HUMAN INSULIN TRANSCRIPTION.

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Background and aims: Human DREAM has been identified as a Ca²⁺-dependent transcriptional repressor. It contains EF hand domains, binds to specific sites in the DNA (the downstream regulatory element DRE) and regulates transcription of c-fos and prodynorphin genes in the central nervous system. The aim of this study was to investigate if DREAM is expressed in cells of the islet of Langerhans and to analyze a possible influence of DREAM on insulin transcription. **Methods:** Tissue expression of DREAM was investigated by Northern blot and immunohistochemical methods. In order to test involvement of DREAM in the transcriptional control a luciferase reporter system with the main part and various truncated versions of the human insulin promoter were employed. **Results:** We demonstrate the expression of DREAM in human pancreatic islets of Langerhans and in the rat insulinoma cell line RIN-5F at protein and mRNA level. Furthermore we identified a DRE site linked to the proximal CRE-element in the human and rat insulin promoters. Using a luciferase reporter vector (pGL3-luc) and the human insulin promoter as well as truncated versions we demonstrate a strong influence of DREAM on insulin transcription in cotransfection experiments. **Conclusions:** DREAM might represent an important insulin transcription regulatory protein in β -cells of the islet of Langerhans which itself is influenced by cytoplasmic and nuclear Ca²⁺-fluctuations. Interestingly, expression of DREAM in β -cells is influenced by secretagogin, a β -cell- and neuroendocrine cell-specific Ca²⁺-binding protein.

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TSC-22 (TGF-beta-stimulated clone-22) Represses the Transcription of Insulin Gene

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In order to identify the candidate genes for type 2 diabetes mellitus in the pancreatic beta-cells, we performed fluorescent differential display with cDNAs derived from the pancreatic islets of 8-week-old GK rats, a model of non-obese type 2 diabetes mellitus, and Wistar control rats. We isolated 34 independent clones showing differentially expression in the pancreatic islets of GK rats. The expression levels of 11 clones among them were down-regulated and the levels of the others were up-regulated in GK rats. Among them, TSC-22 (TGF-beta-stimulated clone-22) was expressed uniquely in the pancreatic islets of GK rat. It was reported that TSC-22 encodes a leucine zipper-containing protein and has transcriptional repressor activity. It was also reported that TSC-22 can homodimerize and heterodimerize with other transcriptional family members. Therefore, we have examined the effects of TSC-22 on the transcriptional activity of insulin promoter. The 5'-flanking sequence of human insulin gene was subcloned into a luciferase expression plasmid. This luciferase reporter plasmid was transfected into mouse derived insulinoma cell line MIN6 cells with or without TSC-22 expression plasmid constructed with rat TSC-22 full-length cDNA and pCMV plasmid. After 48 hours, luciferase activity was measured. The activity of insulin promoter was repressed to 58% of control by the expression of TSC-22. The deletion analysis was performed to reveal the region of insulin promoter that was interacted with TSC-22. It revealed that -170 to -88 of insulin promoter was important for interaction with TSC-22. We conclude that TSC-22 may play a suppressive role in insulin gene transcription in the pancreatic beta-cells of GK rats and also that it may be one of the candidate genes for type 2 diabetes mellitus.

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GENE THERAPY OF PANCREATIC-DERIVED β -CELLS WITH GLP-1 RESTORES GLUCOSE-DEPENDENT INSULIN PRODUCTION.
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Background and Aims: Glucagon-like peptide-1 (GLP-1) is an incretin hormone derived from the proglucagon gene, capable of regulating the transcription of the three major genes that determine the pancreatic β -cell-specific phenotype: insulin, GLUT-2 and glucokinase. The aim of this study was to investigate the potential role of GLP-1 for the gene therapy of a glucose-insensitive pancreatic β -cells. **Materials and Methods:** We transfected mouse insulinoma (MIN-6) cells with a DNA fragment of the human proglucagon gene containing the nucleotide sequence encoding for human GLP-1, but lacking the coding region for glucagon. Two constructs were generated: in one of them, the expression of GLP-1 was under control of the CMV promoter (CMV/GLP-1), while in the second, the rat insulin II promoter (Ins/GLP-1) regulated it. **Results:** Northern blot analysis of the two cell lines revealed that both CMV/GLP-1 and Ins/GLP-1 cells expressed a greater insulin mRNA levels when compared to controls; however while CMV/GLP-1 cells were glucose insensitive, Ins/GLP-1 were capable of regulating insulin and GLP-1 gene expression based on the concentration of glucose in the culture medium. Detection of the counterpart proteins in the culture medium paralleled the observation derived from the northern blot analysis. GLP-1 action was mediated by an IDX-1-dependent transactivation of the endogenous insulin promoter, as demonstrated by gel shift analysis. This was further suggested by a significant increase of the glucose-dependent nuclear translocation of IDX-1 protein in Ins/GLP-1 cells, when compared to CMV/GLP-1 cells or parental MIN-6 cells. Finally, we observed that GLP-1-dependent acquisition of glucose responsiveness was not affected by an increased expression level for GLP-1 receptor. **Conclusions:** In summary, our study provides further evidence that GLP-1 by restoring glucose-dependent insulin production is a unique candidate for the therapy of type 2 diabetes.

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REGULATION OF GLUCOKINASE GENE EXPRESSION IN PANCREATIC B-CELLS BY RETINOIC ACID AND VITAMIN D3

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Background and Aims: Previous analysis of the B-cell active glucokinase gene (bGK) promoter revealed cis-elements, TGGT1 and TGGT2, showing a high homology to binding sites for members of the nuclear receptor superfamily, such as vitamin D3 receptor (VD3R) and retinoic acid receptor (RAR). Here, we studied the involvement of VD3R, RAR and RXR in the regulation of the bGK promoter in pancreatic B-cells.

Materials and Methods: To investigate the effect of RA and VD3 on the bGK expression we performed comparative RT-PCR. bGK promoter (-278/+123) driven reporter gene expression (CAT and GFP) was analysed in transient expression studies. To determine the effect of 1 μ M RA or 10nM VD3 on bGK promoter-driven GFP expression, cells were stimulated for 25 min with either hormone. GFP fluorescence was monitored online over a period of 4 hours. To analyse the role of RAR, RXR and VD3R complexes, these receptors were overexpressed together with the bGK-GFP construct.

Results: Stimulation of islets or HIT cells with RA or VD3 lead to a 1.5 to 3 fold increase in mRNA levels. Online monitoring experiments revealed a 1.6 \pm 0.1- or 1.4 \pm 0.1fold increase in GFP-fluorescence in response to RA- or VD3-stimulation. Mutation of either TGGT1 (at -90bp) or TGGT2 (at -168bp) lead to a drastic reduction in basic expression (to 34% or 28% of wild type, respectively) and to a drastic reduction (in case of TGGT2) or complete loss of response to RA and VD3 stimulation. The hormone-stimulated bGK promoter activity was enhanced following overexpression of VD3R (to 2 \pm 0.2fold) or VD3R/RXR (to 1.7 \pm 0.1fold) or of a combination RAR/RXR (to 2.15 \pm 0.3fold). RAR/RXR had no effect on the VD3-stimulated expression, while VD3R had no effect on RA-stimulated expression.

Conclusion: We demonstrate that the bGK expression in the pancreatic B-cell can be stimulated by VD3 or RA. While both regulatory pathways seem to converge on the same cis-elements, each hormone uses its own distinctive trans-factors, RAR/RXR for RA and VD3R or VD3R/RXR for VD3.

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PROTEOME ANALYSIS OF ISLETS FROM RATS FETUS MALNOURISHED IN UTERO

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Background: Maternal isocaloric protein restriction during pregnancy in rats impairs islet cell proliferation as well as insulin secretion and increases apoptosis in the fetal β -cells. Low protein diet (LP) in early life also increases susceptibility of fetal β -cells to nitric oxide (NO) and IL-1 β . **Aim:** To identify changes in protein expression levels in islets from fetuses subjected to LP during gestation. **Methods:** Pregnant Wistar rats were fed a LP diet (8% protein) or a control diet (20% protein) throughout gestation. At day 21.5 of gestation islets were isolated. During 7 days of culture fetal islets were neoformed. Fetal islets were labelled with [³⁵S]-methionine for two-dimensional gel electrophoresis. Each sample was run on isoelectric focusing (IEF, pH 3.5-7) and non-equilibrium pH-gradient electrophoresis (NEPHGE, pH 6.5-10.5) and analyzed on a BioImage computer program. Changes in expression levels of proteins were expressed as percentage of integrated optic density and considered significant at $p < 0.01$ (Student's t-test). Significantly changed spots were identified by mass spectrometry. **Results:** In total 2.810 protein spots were identified on the gels. Seventy of these spots were significantly changed in LP islets compared to control islets. On the IEF side 1.916 spots whereof 55 were significantly changed and on the NEPHGE side 894 spots whereof 15 were significantly changed compared to control diet. So far we have 5 identifications (elongation factor 1 α , HSC70-PS1, WDR1 protein, albumin and fuse binding protein 2), 3 good spectra but no identification and 3 with too few peptides. **Conclusion:** LP diet during development significantly change the expression level of 70 spots out of 2.810 protein spots compared to control diet. These proteins might be involved in the altered β cell mass and the increased susceptibility to NO and cytokines in LP islets and therefore of potential interest for type 1 diabetes mellitus pathogenesis.

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MICROARRAY STUDIES OF ANGIOGENIC FACTORS AND CHEMOKINES EXPRESSED IN MICROENCAPSULATED ISLETS OF LANGERHANS.

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Background and Aims: Microencapsulation of islets of Langerhans may provide the means to transplant islets in the absence of immunosuppression. However, little is known about the cell biology of microencapsulated islets. An increased expression of chemokines in microencapsulated islets could induce an immune response against the microencapsulated islets and angiogenic factors may be expressed in response to hypoxia. The aim of this project was to study the mRNA expression of chemokines and angiogenic factors in microencapsulated islets of Langerhans. **Materials and Methods:** Islets were isolated from Balb/c mice and after 5-7 days culture, microencapsulated in alginate/poly-L-lysine/alginate capsules. Microencapsulated or free islets were then cultured for an additional 1-week period. Alternatively, microencapsulated islets were either syngeneically transplanted or cultured for 3 days. To isolate RNA, islets were removed from the capsules, by means of microdissection, and lysed in lysis buffer. RNA was then isolated using RNeasy kit. A microarray kit (GeArray) was used to study the mRNA expression of a variety (23-24) of chemokines and angiogenic factors, which were compared with beta-actin and glyceraldehyde-3-phosphatase dehydrogenase (GAPDH) expression. **Results:** The most abundant chemokines detected after 1-week culture were macrophage inflammatory protein 1 α (MIP-1 α), and to a lesser extent, I-309 (or scya 1; small inducible cytokine A1 precursor), both of which were expressed at similar levels in free and microencapsulated islets. A variety of angiogenic factors were detected to similar extents in free and microencapsulated islets after 1-week culture. These included tyrosine kinase receptor 1 (Tie 1), vascular endothelial growth factor (VEGF), angiogenin, angiopoietin and the anti-angiogenic factor endostatin. However, transplanted microencapsulated islets had lower expression of VEGF, Tie 1 and angiopoietin compared with cultured microencapsulated islets. **Conclusions:** Islet expression of a variety of chemokines and angiogenic factors was not altered by microencapsulation. However, the expression of some angiogenic factors was decreased after transplantation of microencapsulated islets.

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Metabolic Signalling in Beta-Cells

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ASSESSMENT BY D-[³H]MANNOHEPTULOSE UPTAKE OF B-CELL DENSITY IN ISOLATED ISLETS FROM GOTO-KAKIZAKI RATS

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Background and Aims: The uptake of D-[³H]mannoheptulose, as mediated mainly by GLUT2, was recently proposed as a tool to assess the relative contribution of insulin-producing cells to the total mass of isolated islets. This procedure was so far only used, however, in a comparison between control rats and animals injected with streptozotocin a few days before the experiments. In the present study, the validity of this novel approach was further explored by comparing the uptake of D-[³H]mannoheptulose by islets isolated from either control rats or hereditarily diabetic Goto-Kakizaki rats (GK rats). **Materials and Methods:** Pancreatic islets isolated by the collagenase procedure from fed female Wistar rats or GK rats were incubated for 60 min at 37°C in groups of 20 islets each in 0.1 ml of a salt-balanced medium containing 8.3 mmol/l D-glucose, 2.0 mmol/l sucrose and 0.1 mmol/l D-mannoheptulose mixed with tracer amounts of [U-¹⁴C]sucrose and either ³HOH, D-[5-³H]glucose or D-[³H]mannoheptulose. The islets were then separated from the incubation medium by centrifugation through a layer of oil. **Results:** The extracellular [U-¹⁴C]sucrose and intracellular [³HOH] spaces were not significantly different in control and GK rats. The intracellular D-[5-³H]glucose space was slightly lower in GK rats (1.83 ± 0.09 nl/islet; $n = 16$; $P < 0.05$) than in control animals (2.12 ± 0.11 nl/islet; $n = 16$). The intracellular D-[³H]mannoheptulose space was much lower in GK rats (1.07 ± 0.06 nl/islet; $n = 15$; $P < 0.005$) than in control animals (1.51 ± 0.11 nl/islet; $n = 16$). **Conclusions:** The present data are in close agreement with those of a prior morphometric study indicating that the density of B-cells in isolated islets from adult GK rats represents only 78.5 ± 2.5 % of the mean corresponding control value (100.0 ± 1.3 %).

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POTENTIATION BY METHYL PYRUVATE OF GLP-1 INSULINOTROPIC ACTION.

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Background and Aims: Methyl pyruvate (MP) stimulates insulin release both in vivo and in vitro. The present study aimed at investigating whether MP is able to enhance the B-cell secretory response to glucagon-like peptide (GLP-1). **Materials and Methods:** Fed male Wistar rats anaesthetised with pentobarbital received intravenously either saline or MP diluted in saline (2 μ mol/g body wt in 30 s, followed by 1 μ mol/g per min to min 15). Either saline or GLP-1 in saline (5 pmol/g) was injected 5 min after the onset of the test. Plasma insulin and glucose concentrations were measured in blood samples collected from a carotid artery. Data are presented as mean \pm SEM. The statistical significance of difference between mean values was assessed by Student's t-test. **Results:** Two to five min after injection and subsequent infusion of MP, the plasma insulin concentration was increased by 2.70 ± 55 ng/ml ($n = 18$), as compared ($p < 0.001$) to a paired change of -0.14 ± 0.28 ng/ml ($n = 16$) in the control experiments. The integrated GLP-1 induced increment in plasma insulin concentration (min 5 to min 20 inclusive) above the pair value recorded at the 5th min of the test, just before GLP-1 injection, averaged, in the presence of MP, 102.8 ± 24.4 (ng \times min)/ml, as compared to only 22.6 ± 2.2 (ng \times min)/ml in the absence of MP ($n = 4$ in both cases, $p < 0.02$). In these experiments, MP increased the plasma glucose concentration by 0.52 ± 1.13 and 2.72 ± 0.59 mmol/l ($n = 9$ and $p < 0.05$ in both cases) at min 5 and 20, respectively, whilst no significant change in plasma glucose concentration was recorded in the absence of MP. Nevertheless, the insulinogenic index (paired ratio between plasma insulin and glucose concentration) was significantly higher in the presence than in the absence of MP, both at min 5 (486 ± 98 vs 172 ± 29 μ g/mol, $n = 8-9$, $p < 0.02$) and 2 and 5 min after injection of GLP-1 (2.31 ± 0.31 vs 0.77 ± 0.13 mg/mol, $n = 8$, $p < 0.001$). **Conclusions:** These findings indicate that, under the present experimental conditions, MP acts as both an insulin secretagogue and gluconeogenic precursor, and potentiates the B-cell secretory response to GLP-1.

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DISSOCIATED EFFECTS OF CYTOCHALASIN B AND D ON GLUCOSE TRANSPORT, METABOLISM AND INSULINOTROPIC ACTION IN ISOLATED RAT ISLETS

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Background and Aims: Cytochalasin B inhibits glucose metabolism, but enhances glucose-induced insulin release in isolated islets. The significance of these findings was investigated by comparing the effects of cytochalasin B and D upon glucose transport, catabolism and insulinotropic action in rat islets. **Materials and Methods:** The distribution space of 3-O-methyl-D-[U-¹⁴C]glucose was corrected for that of [U-¹⁴C]sucrose, both expressed relative to the paired ³HOH space, in islets separated from the incubation medium by centrifugation through an oil layer. D-[5-³H]glucose utilization, D-[U-¹⁴C]glucose oxidation and insulin release were measured over 90 min incubation at 37°C. **Results:** Over 5 or 15 min incubation, cytochalasin B, but not cytochalasin D (both 0.02 mmol/l), decreased by 32-37 % the intracellular distribution space of 3-O-methyl-D-[U-¹⁴C]glucose. Cytochalasin B also decreased D-[5-³H]glucose utilization and, to a lesser extent, D-[U-¹⁴C]glucose oxidation, whilst cytochalasin D failed to affect the catabolism of D-glucose. Yet, cytochalasin D was slightly but significantly less efficient than cytochalasin B in augmenting glucose-stimulated insulin output, whether at 8.3 or 16.7 mmol/l D-glucose and whether in the absence or presence of forskolin (10 μ mol/l). Such was not the case, however, when comparing the effect of cytochalasin B and D upon insulin release evoked by non-glucidic nutrients, such as 2-ketoisocaproate or the association of L-leucine and L-glutamine (all 10 mmol/l). **Conclusions:** The dissociated effects of cytochalasin B and D upon the transport and further metabolism of D-glucose and its insulinotropic action suggest a so-far-unidentified interference of cytochalasin B with the B-cell glucose-sensing device.

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SITE-DIRECTED MUTATIONS IN THE FAD-BINDING DOMAIN OF GLYCEROPHOSPHATE DEHYDROGENASE : CATALYTIC DEFECTS WITH PRESERVED MITOCHONDRIAL ANCHORING OF THE ENZYME IN TRANSFECTED COS-7 CELLS

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Background and Aims: Single strand conformation polymorphism analysis of mitochondrial FAD-linked glycerophosphate dehydrogenase (mGDH) gene has revealed mutations in both the calcium- and FAD-binding domains of this enzyme in some diabetic patients. It was now investigated whether site-directed mutations in the FAD-binding domain of the mGDH gene may affect the mitochondrial anchoring and catalytic activity of the enzyme. **Materials and Methods:** COS-7 cells were transfected with plasmid cDNA coding for either wild type or mutated human mGDH (G \rightarrow A substitutions at positions 352, 355 and 364 and A \rightarrow C substitution at position 390) fused at the N-terminus of green fluorescence protein. The activity of mGDH was measured by both a radioisotopic (³HOH production from L-[2-³H]glycerol-3-phosphate) and colorimetric (iodoformazan formation) procedure. **Results:** In cells transfected with the mGDHwt-EGFP or mGDHmut-EGFP constructs, the fused protein was found by confocal microscopy exclusively in the mitochondria, colocalized with a mitochondrial marker. In homogenates of COS-7 cells transfected with mGDHmut, however, the catalytic activity of the enzyme was decreased, this coinciding with low ratios between both the activities measured in the absence/presence of exogenous FAD and the results obtained by the colorimetric/radioisotopic procedure. **Conclusions:** Although the present site-directed mutations of the mGDH gene failed to impair the mitochondrial anchoring of the enzyme, they led to catalytic defects that were, in some respect, comparable to those previously encountered in the lymphocytes or islets of type-2 diabetic patients.

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Mitochondrial metabolism sets the upper limit of fuel-stimulated insulin secretion: Evaluation by computer simulation and engineered triose-stimulated insulin secretion

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Background and Aims: We have recently developed a computer model of beta-cell metabolism to test theories of metabolism secretion coupling. Agreeing with published data on the high control strength of glucokinase, this computational model demonstrates that glycolytic metabolites and mitochondrial oxidation increase with increasing glucose concentrations until reaching a plateau around 20mM glucose. Since glycolytic and mitochondrial pools of metabolites increase similarly, it is difficult to discern their individual roles on insulin secretion. Our goal in this study is to discriminate the importance of these two metabolite pools in metabolism secretion coupling.

Materials and Methods: In silico experiments were performed with the CLOCCS four-compartment beta-cell model. In vitro experiments were performed in INS-1 cells treated with glycerol kinase adenovirus. Metabolites were measured by spectrophotometric enzymatic techniques and mitochondrial membrane potential by rhodamine-123 fluorescence. Mitochondrial calcium was measured with aequorin luminescence and insulin secretion was monitored simultaneously from a perfusion setup.

Results: Utilizing computer simulations, we devised a strategy to increase the triose pool (DHAP, GlcP) above levels achieved with a maximally stimulatory glucose concentration. Expression of glycerol kinase was selected since beta-cells are permeant to glycerol and express suitable levels of glycerol phosphate dehydrogenase necessary to drive glycerol phosphate into the lower half of glycolysis. Transducing INS-1 cells with the glycerol kinase adenovirus and using 2mM glycerol achieves a triose concentration of 295 \pm 28 μ M compared to 112 \pm 40 μ M with 16mM glucose. Fructose 1,6 BP levels are >4-fold higher with 2mM glycerol (1287 \pm 202 μ M) versus 16mM glucose (305 \pm 175 μ M). Cells treated with 2mM glycerol secrete 3 times more lactate than cells treated with 16mM glucose. Despite these large increases in glycolytic metabolites, insulin output is no greater with 2mM glycerol compared to 16mM glucose. We therefore evaluated if mitochondrial metabolism limits insulin secretion. Mitochondrial membrane potential is similar between 2mM glycerol and 16mM glucose treatment as is the peak mitochondrial calcium concentration, 768 \pm 196nM versus 792 \pm 218nM, respectively.

Conclusions: Based on these observations, we conclude that the capacity of mitochondrial metabolism sets the upper limit of fuel-mediated insulin secretion.

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DOES GLUTAMATE CONVERSION TO GABA PREVENT THE STIMULATION OF INSULIN SECRETION BY L-GLUTAMINE IN ISLETS?

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Background and Aims: It is generally assumed that the allosteric activation of islet glutamate (Glu) dehydrogenase by L-leucine increases L-glutamine (Gln) metabolism in the Krebs cycle, thereby stimulating insulin secretion.

Materials and Methods: We have investigated the effects of 10 mmol/l L-leucine (Leu) on ¹⁴C¹⁴O₂-production from L-[U-¹⁴C]glutamine (pmol/ μ g protein) and on islet amino acids (pmol/h \times μ g protein) by means of their derivatization with o-phthalaldehyde and HPLC-separation of the fluorescent derivatives.

Results: [U-¹⁴C]Gln conversion to ¹⁴C¹⁴O₂ followed a hyperbolic relationship with the concentration (0.1 to 10 mmol/l) and the half-maximal rate was reached at 0.5 mmol/l. Gln was also converted to other amino acids: half-maximal levels of GABA were reached around 0.3 mmol/l and those of Asp and Glu at 0.5 and 1.0 mmol/l, respectively. Leu stimulated a predominantly monophasic release of insulin which became biphasic in the presence of 10 mmol/l Gln (20.3 \pm 1.5, n=41, vs. 9.8 \pm 1.8, n=13, p<0.001). This secretory synergism between Leu and Gln was suppressed by 10 mmol/l malonic acid dimethyl ester (a competitive inhibitor of succinic acid dehydrogenase) (9.5 \pm 1.8, n=6, vs. 20.3 \pm 1.5, n=41, p<0.01). Leu decreased the islet content of Gln (55.6 \pm 3.5, n=6, vs. 73.9 \pm 3.3, n=6, p<0.01) and GABA (43.5 \pm 3.4, n=6, vs. 70.6 \pm 4.2, n=6, p<0.001) in islets incubated with 10 mmol/l Gln but it did not modify the rate of ¹⁴C¹⁴O₂-production from 10 mmol/l [U-¹⁴C]Gln. At 0.5 mmol/l Gln, the rate of ¹⁴C¹⁴O₂-production was significantly decreased by Leu either in the absence (19.9 \pm 1.4, n=8, vs. 30.4 \pm 1.6, n=21; p<0.001) or presence (16.1 \pm 1.1, n=4, vs. 27.5 \pm 2.4, n=4, p<0.01) of 2 μ g/ml oligomycin. The latter did not exert any significant effect. Leu diminished significantly the intracellular contents of Glu (22.6 \pm 1.4, n=6, vs. 31.7 \pm 2.0, n=6, p<0.01), Gln (3.3 \pm 0.4, n=6, vs. 5.5 \pm 0.8, n=6, p<0.05) and GABA (22.2 \pm 1.7, n=6, vs. 45.3 \pm 4.5, n=6, p<0.001) in islets incubated with 0.5 mmol/l Gln.

Conclusions: 1. [U-¹⁴C]Gln conversion to ¹⁴C¹⁴O₂ was not sensitive to oligomycin and it was mostly accounted for by islet GABA accumulation (Glu-decarboxylation). 2. Leu induced a parallel decrease of islet GABA accumulation and ¹⁴C¹⁴O₂-production from [U-¹⁴C]Gln. 3. It is proposed that inhibition of GABA synthesis by Leu, together with its well known activation of glutamate dehydrogenase, diverts more Glu into the Krebs cycle and this accounts for the observed secretory synergism between Leu and Gln.

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AN OMEGA-6 LIPOXYGENASE FATTY ACID METABOLITE UPREGULATES THE NUMBER OF READILY RELEASEABLE INSULIN GRANULES

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Background & Aims: Metabolism of the essential fatty acids (EFA) linoleate (omega-6) and linolenate (omega-3) is suggested to be important for adaptation of insulin secretion under various conditions. Arachidonic acid (AA) is synthesised via the omega-6 pathway and is further metabolised by lipo- & cyclooxygenases. Here, we have elucidated the role of omega-6 EFA for B-cell electrical activity, single-cell release kinetics and insulin secretion.

Materials & Methods: Female Sprague Dawley rats were fed with a semi-synthetic diet without either omega-6 EFA. Insulin release from isolated islets in static incubations was measured using RIA. Single B-cell electrical activity was monitored using the patch clamp technique combined with capacitance recordings of exocytosis.

Results: Glucose-stimulated insulin secretion was suppressed by ~30% in omega-6-deficient (O6D) rat islets, in spite of normal glucose-induced inhibition of ATP-sensitive K-channels and even slightly accelerated action potential firing rate in B-cell clusters from O6D rats. The latter effect could be explained by voltage-gated Ca²⁺-influx being almost 3-fold potentiated. Integrated Ca²⁺ currents elicited by single 500 ms depolarisations averaged 28 \pm 10 and 85 \pm 11 pA in O6D and control cells, respectively (P<0.001; n=15 [O6D] & 12 [control]). However, depolarisation-evoked exocytosis in O6D B-cells averaged only 38% of that observed in normal cells (P<0.05; n=8 [O6D] & 9 [control]). Whereas a single depolarisation releases the readily releasable pool of insulin granules, intracellular dialysis of a Ca²⁺-containing pipette solution elicits sustained exocytosis that involves recruitment of new insulin granules for release. This process was likewise inhibited by ~60% in O6D rats (P<0.01; n=12 [O6D] & 15 [control]). The O6D-induced suppression of exocytosis was fully counteracted by either linoleic or arachidonic acid (5 μ M). By contrast, neither excess omega-3 or the non-metabolisable AA analogue ETYA (10 μ M) could reconstitute exocytosis. The AA-mediated potentiation of exocytosis was prevented by the 12-lipoxygenase inhibitor esculetin (0.5 μ M). In addition, the AA stimulatory action depended on protein kinase C and was abolished by bisindolylmaleimide.

Conclusions: An omega-6 lipoxygenase metabolite is crucial for B-cell function by: 1) upregulating Ca²⁺-induced insulin release via a PKC-dependent mechanism, and 2) making Ca²⁺-signalling more efficient. These results favour the ideas that omega-6 metabolism plays a role for enhancing GSIS with increasing insulin resistance and for protection against type 1 diabetes.

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CARBONIC ANHYDRASE ACTIVITY IN PANCREATIC ISLETS

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Background and Aims: Carbonic anhydrase isoenzymes may participate in nutrient-induced insulin release by coupling the exchange between influent CO₃H⁻ and effluent Cl⁻ to the intracellular conversion of CO₃H⁻ and H⁺ to CO₂ and H₂O, by providing bicarbonate for mitochondrial pyruvate carboxylase and subsequent circulation in the pyruvate-malate shuttle and/or by regulating mitochondrial Ca²⁺ concentration. The expression of carbonic anhydrase in islet B-cells was recently documented by both immunohistochemical staining and Western blot analysis. We have now measured carbonic anhydrase activity in islet homogenates. **Methods:** Carbonic anhydrase activity was judged from the rate of ¹⁴CO₂ production from ¹⁴CO₃H⁻, as catalyzed by islet or parotid cell homogenates. **Results:** In islet homogenates, the generation of ¹⁴CO₂ from ¹⁴CO₃H⁻ was proportional to incubation time (3-9 min), bicarbonate concentration (0.05-0.5 mmol/l) and islet number (30-60 islets). At an 0.5 mmol/l bicarbonate concentration, it averaged 2.6 \pm 0.6 pmol.min⁻¹ per μ g protein, representing about 25 % of the value in parotid cell homogenates, in which carbonic anhydrase represents an export protein. Acetazolamide inhibited enzymic activity in both islet and parotid homogenates, but differences in concentration dependency suggested the participation of distinct isoenzymes. In intact islets, acetazolamide (0.1-5.0 mmol/l), although causing a progressive increase in the ratio between D-[U-¹⁴C]glucose oxidation and D-[5-³H]glucose utilization, progressively decreased insulin output evoked by D-glucose (16.7 mmol/l). **Conclusions:** The present findings reveal that carbonic anhydrase activity in islet homogenates would be sufficient to ensure full conversion of glucose-derived CO₂ to bicarbonate, and indeed suggest that integrity of the enzyme activity is required to ensure a normal secretory response of the islet B-cells to D-glucose.

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A NMR STUDY OF GLUCOSE AND ALANINE METABOLISM BY THE BETA CELL LINES RINm5F AND BRIN-BD11.

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Background and Aims: Despite many studies of glucose and amino-acid induced insulin secretion little is known about the relative importance of the various routes of metabolism of these secretagogues. A study was designed to identify the key metabolic end-products of beta-cell glucose and alanine metabolism. Although it is clear that metabolism of glucose is required for insulin secretion it is not yet clear whether metabolism of alanine is required for the stimulatory effect of this amino acid on insulin secretion.

Materials and Methods: BRIN-BD11 and RINm5F cells were grown in RPMI media supplemented with 10 % FCS, 2mM glutamine and antibiotics. Cells were subsequently incubated in monoculture at a density of 0.7-0.8x10⁵ cells per 60mL KRB buffer pH 7.4 which was supplemented with either 10mM 3-13C labelled alanine or 16.7mM 1-13C labelled glucose. The cells were extracted with 6 % PCA and debris removed from the flask using a cell scraper. After centrifugation the cell extracts were neutralised with KOH. The resulting supernatant was freeze dried. A NMR sample was then prepared and 13C NMR spectra were acquired using either a Varian Unity Plus 400 MHz spectrometer or a Bruker DRX 500 MHz spectrometer.

Results: Identification of key metabolites was achieved using 13C NMR. When beta-cells were incubated in the presence of 16.7mM glucose the NMR detectable metabolic end-products included lactate, acetate, glutamate and alanine. When beta-cells were incubated in 10mM alanine the NMR detectable metabolic end-products included acetate, lactate, glutamate and aspartate. When both glucose and alanine were included in the incubation medium the presence of alanine dramatically enhanced all aspects of glucose metabolism.

Conclusions: We detected similar key end-products of glucose metabolism by RINm5F and BRIN-BD11 cells. It has been suggested that Na⁺-alanine co-transport is responsible for alanine induced insulin secretion via depolarisation of the plasma membrane. However, we have demonstrated significant metabolism of alanine by RINm5F and BRIN-BD11 cells which may be related to the enhanced rates of insulin secretion observed in the presence of this amino acid. Future work will involve determining the impact of the rate of generation of key end-products of metabolism, to rates of insulin secretion from the intact beta-cell cultured at high density in a purpose built bioreactor.

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ENZYMATIC ACTIVITIES IN TWO POPULATIONS OF PURIFIED RAT ISLET B-CELLS

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Background and Aims: In terms of glucose sensing by islet B-cells, emphasis is often placed on both the role of glucokinase, with negligible activity of low-Km hexokinase(s), and the prevalence of the oxidative modality of glycolysis, a situation attributed by certain authors to a low lactate dehydrogenase activity. Conflicting information is available, however, on the activity of both low-Km hexokinase(s) and lactate dehydrogenase in B-cell homogenates. This issue was reinvestigated, therefore, in two populations of purified rat islet B-cells selected on the basis of their low (BL) or high (BH) content in NAD(P)H. **Materials and Methods:** Rat islet B-cells were identified by their Fluo3 fluorescence and forward scatter of light. Their NAD(P)H fluorescence at 16 mmol/l D-glucose was simultaneously measured, and the cells in the lower and upper tertiles of NAD(P)H signal intensity sorted as the BL and BH cells. **Results:** The mean size, protein content and activity of low-Km hexokinase, lactate dehydrogenase, mitochondrial glycerophosphate dehydrogenase, glutamate dehydrogenase, glutamate-alanine transaminase and glutamate-aspartate transaminase was about twice higher in BH than BL cells. The activity of glucokinase was comparable, however, in BL and BH cells. Even in BL cells, the activity of low-Km hexokinase at 1.0 mmol/l D-glucose was not lower than that of glucokinase at 10.0 mmol/l D-glucose. Likewise, even in BL cells, the activity of lactate dehydrogenase, although being assessed from the unfavourable rate of L-lactate conversion to pyruvate and when expressed as D-glucose equivalent, was not significantly lower than that of glucokinase at 10.0 mmol/l D-glucose. **Conclusions:** A low expression of low-Km hexokinase(s) and lactate dehydrogenase, as postulated by other authors, is not evident in purified rat islet B-cells.

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K_{ATP} CHANNELS ARE PRESENT BUT DO NOT SEEM TO BE INVOLVED IN THE STIMULATION OF MOUSE PANCREATIC ALPHA-CELLS

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Background and aims: Relatively little is known about the signal transduction underlying glucagon secretion. We now study the effect of natural stimuli like adrenaline and amino acids on the cytoplasmic Ca²⁺ concentration ([Ca²⁺]_i), which controls glucagon release from the pancreatic α-cell.

Materials and Methods: Single cells were prepared from pancreatic islets of normal mice.

[Ca²⁺]_i was measured with a digital imaging technique and the cells were then identified by immunostaining.

Results: In the presence of 3 mmol/l glucose no insulin-releasing β-cells and only about 5% of the α-cells exhibited spontaneous activity with large amplitude oscillations of [Ca²⁺]_i in the 0.2-0.5 /min range. Previously silent α-cells but not β-cells were found to react to adrenaline and low concentrations of glutamine and glycine with elevation of [Ca²⁺]_i; often manifested as oscillations. In contrast practically all β-cells but only about 6 % of the α-cells showed a [Ca²⁺]_i response to tolbutamide, which inhibit ATP-dependent (K_{ATP}) channels. The K_{ATP} channel activator diazoxide and an inhibitor of voltage-dependent Ca²⁺ channels prevented the maintained α-cell response to adrenaline and amino acids, but the inhibitory action of diazoxide was reversed by tolbutamide. Nevertheless, diazoxide did not preclude the initial [Ca²⁺]_i elevation of in response to adrenaline. The adrenaline effect was mimicked by forskolin and antagonised by inhibitors of protein kinase A as well as by the β-adrenergic antagonist propranolol.

Conclusions: Mouse pancreatic α-cells have K_{ATP} channels although their function is obscure. Adrenaline and amino acids act by raising [Ca²⁺]_i via influx through voltage-dependent channels. The adrenaline effect involves rise of cAMP and mobilisation of intracellular Ca²⁺.

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ROLE OF THE SARCO-ENDOPLASMIC RETICULUM Ca^{2+} -ATPASE SERCA3 IN Ca^{2+} SIGNALING IN MOUSE β -CELLS.

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Pancreatic islets express both the high Ca^{2+} -affinity SERCA2b and the low Ca^{2+} -affinity SERCA3. Dysregulation of the SERCA3 activity has been associated with type 2 diabetes in mice and humans. Wild type (+/+) and SERCA3 KO (-/-) mice were used to evaluate the role of SERCA3 in β -cell $[\text{Ca}^{2+}]_i$ regulation and insulin secretion, and to estimate its contribution to glucose homeostasis. **Methods.** $[\text{Ca}^{2+}]_i$ was measured (fura-PE3) in isolated islets or clusters of islet cells, and insulin secretion was monitored from perfused islets. **Results.** Stimulation with 15 mmol/l glucose (G) induced an initial, transient drop in $[\text{Ca}^{2+}]_i$ ascribed to pumping into the endoplasmic reticulum (ER) because of its suppression by thapsigargin (TG). This drop was unaffected by SERCA3 ablation. Mobilization of intracellular Ca^{2+} by acetylcholine or TG was similar in +/+ and -/- β -cells. $[\text{Ca}^{2+}]_i$ oscillations induced by glucose or pulses of high K^+ normally display a slow descending phase caused by slow Ca^{2+} release from the ER. SERCA3 ablation (like TG) increased the peak of $[\text{Ca}^{2+}]_i$ during the oscillations and accelerated the descending phase. This indicates that Ca^{2+} released by the ER into the cytosol at the end of each oscillation was pumped into the ER by SERCA3 during the upstroke phase. Average $[\text{Ca}^{2+}]_i$ at 3 to 20 mmol/l G was similar in +/+ and -/- β -cells. Biphasic insulin secretion in response to 15 mmol/l was not smaller in -/- than +/+ islets. Refeeding after 24h fasting did not reveal impaired glucose homeostasis in -/- mice. **Conclusions** SERCA2b pumps Ca^{2+} into the ER at basal $[\text{Ca}^{2+}]_i$ in β -cells. SERCA3 becomes operative when $[\text{Ca}^{2+}]_i$ is elevated during $[\text{Ca}^{2+}]_i$ oscillations. SERCA3 expression is not required for normal insulin secretion and glucose homeostasis, and its defective expression does not contribute to development of type 2 diabetes as previously suggested.

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THE HUMAN PEPTIDE, α -ENDOSULFINE, DIRECTLY BLOCKS L-TYPE Ca^{2+} CURRENTS IN MIN6 β -CELLS.

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Background and Aims: α -Endosulfine, a 121-amino acids 13-kDa protein which belongs to the cAMP-regulated-phosphoprotein family, was originally isolated as an endogenous ligand for the sulfonylurea receptor. α -Endosulfine has been shown to displace sulfonylurea binding, block ATP-sensitive K^+ -channels activity (KATP) and stimulate insulin secretion at basal glucose concentrations. At stimulatory glucose concentration, α -endosulfine inhibits insulin secretion and calcium influx. This inhibition, independent of KATP blockade, is observed on MIN6 β -cells and isolated perfused rat pancreas. We have investigated the electrophysiological mechanism underlying this inhibitory effect.

Materials and Methods: Using the perforated-patch whole-cell patch-clamp technique, we have recorded on MIN6 cells the effects of α -endosulfine on membrane potential and calcium currents. Each value is quoted as mean \pm s.e.m.

Results: In the absence of glucose, the membrane potential (V_m) of the MIN6 cell was electrically silent at -67 ± 1 mV ($n=16$). Under these conditions, the addition of 1 μM α -endosulfine resulted in a rapid depolarization of the membrane potential to a steady-state value of -19 ± 6 mV ($n=3$) and a transient firing of action potentials. The presence of 10 mM glucose depolarized the MIN6 membrane potential to -51 ± 2 mV and induced action potential firing. Subsequent addition of 1 μM α -endosulfine further depolarized V_m to -20 ± 3.9 mV ($n=7$); this was associated with the abolition of action potentials. These effects were entirely reversed on removal of the peptide. Similar experiments performed with 200 μM tolbutamide showed that the sulfonylurea did not mimic the effect of α -endosulfine, but instead produced a small depolarization which was associated with a maintained stimulation of electrical activity. Under voltage-clamp, characteristic inward Ca^{2+} -currents flowing through L-type Ca^{2+} -channels were elicited at potentials positive to -60 mV from holding potential of -70 mV. At 0 mV, 1 μM α -endosulfine blocked the peak current by $40 \pm 2.3\%$ and the steady-state current by $85 \pm 4\%$ ($n=4$). The block was independent of both use and voltage.

Conclusions: The primary event occurring upon addition of α -endosulfine to MIN6 cells subjected to glucose stimulation, is inhibition of L-type Ca^{2+} -channels. α -Endosulfine acts as an open Ca^{2+} -channel blocker reducing Ca^{2+} influx and consequently insulin secretion.

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Impaired transport of insulin secretory granules in the rat insulinoma cell line INS-1 expressing antisense Ca^{2+} /calmodulin dependent protein kinase II d2 (CaM Kin II d2)

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Introduction: CaM Kin II d2 is supposed to play a role in stimulated insulin secretion. Synapsin I mediated actin web debundling after phosphorylation by CaM Kin II d2 is one mechanism thought to be involved in the regulated refilling of the so called readily releasable pool of insulin granules. We had shown a strong association between insulin secretory granules and CaM Kin II d2 by means of subcellular fractionation and immunofluorescence as well as coprecipitation of synapsin I and CaM Kin II d2 in previous works. An INS-1 cell line overexpressing antisense CaM Kin II d2 showed a decrease in CaM Kin II expression.

Aim: It was the aim of this study to investigate the distribution of insulin granules in insulinoma cells overexpressing CaM Kin II d2 by confocal microscopy.

Methods: INS-1 cells overexpressing antisense CaM Kin II d2 were grown in RPMI 1640 medium under standard conditions. Confocal laser immunofluorescence was done with a Zeiss laser scanning microscope. Visualization was performed with a anti-insulin antibody and anti-mouse secondary antibody purchased from sigma

Results: Confocal laser immunofluorescence of antisense cells showed a pattern of insulin granule distribution distinct from wt INS-1 cells. In contrast to the regular submembraneous pattern of insulin secretory granules in INS-1 there was a perinuclear pattern of insulin secretion granules in CaM Kin II d2 antisense expressing cells.

Discussion: These findings point toward a role of CaM Kin II d2 in the modulation of insulin granule transport to the plasma membrane. The transport defect may be the consequence of decreased synapsin I phosphorylation due to CaM Kin II d2 antisense expression.

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Palmitate potentiates glucose-induced insulin secretion by increasing cytoplasmic Ca^{2+} and electrical activity in mouse B-cells

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Background and Aims: It is well established that acute exposure of pancreatic islets to free fatty acids leads to stimulation of insulin release. We have correlated the effects of palmitate on insulin secretion to changes of the cytoplasmic free Ca^{2+} ($[\text{Ca}^{2+}]_i$) with the goal of identifying the participating mechanisms.

Materials and Methods: Collagenase-isolated intact mouse pancreatic islets were used throughout. Changes in $[\text{Ca}^{2+}]_i$ were recorded in fura-2 loaded islets using microfluorimetry. Insulin secretion was assessed by radioimmunoassay. Electrical activity was measured applying the perforated patch whole-cell configuration to B-cells in intact islets. Palmitate was applied at a concentration of 1 mM and was dissolved with 1% fatty acid free bovine serum albumin.

Results: Palmitate had no effect on basal insulin release measured at 3 mM glucose. Adding palmitate to islets exposed to 7.5 or 15 mM glucose increased insulin secretion by 40% and 85% respectively ($n=8$). The amount of insulin released in the simultaneous presence of 7.5 mM glucose and palmitate approached that obtained in response to 15 mM glucose alone. In islets exposed to 15 mM glucose, $[\text{Ca}^{2+}]_i$ oscillated between a plateau level and discrete peaks. The frequency of these oscillations (peak-to-peak) was 2.2 ± 0.58 min⁻¹. Addition of 1 mM palmitate slightly ($\pm 15\%$) elevated the plateau concentration and increased the frequency of the oscillations to 3.2 ± 0.46 min⁻¹ ($+45\%$). Occasionally (20%), addition of palmitate suppressed the oscillatory pattern and produced a sustained elevation of $[\text{Ca}^{2+}]_i$. $[\text{Ca}^{2+}]_i$ rarely oscillated in islet exposed to the subthreshold glucose concentration 7.5 mM but oscillations similar to those seen at 15 mM glucose were then inducible by addition of palmitate. These effects of palmitate on $[\text{Ca}^{2+}]_i$ and insulin secretion correlated with stimulation of glucose-induced (15 mM) electrical activity and the normal bursting pattern was replaced by uninterrupted action potential firing similar to what is observed at glucose concentrations ≥ 20 mM.

Conclusions: We conclude that the ability of palmitate to stimulate insulin secretion involves stimulation of B-cell electrical activity and elevation of $[\text{Ca}^{2+}]_i$. We further propose that the stimulatory action is secondary to increased generation of ATP, which promotes insulin secretion by both closing KATP-channels (thus increasing electrical activity and Ca^{2+} -entry) and providing the metabolic energy required for insulin exocytosis and/or mobilisation of new granules for release.

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DIVERSE ACTIONS OF PROTEIN KINASE C IN CARBACHOL-INDUCED Ca^{2+} SIGNALING IN MOUSE BETA-CELLS

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Background and Aims: The neurotransmitter acetylcholine (ACH) increases $[\text{Ca}^{2+}]_i$ by activating phosphatidylinositol breakdown thereby generating IP_3 and diacylglycerol, which activates protein kinase C (PKC). In the present study the potential role of PKC for the generation of ACH-linked Ca^{2+} -signals was investigated. **Materials and Methods:** $[\text{Ca}^{2+}]_i$ was measured in single fura-2 loaded beta-cells of NMRI mice. **Results:** The ACH analogue carbachol (3 μM) increased $[\text{Ca}^{2+}]_i$ by $226 \pm 45 \text{ nM}$ ($n = 25$). Pretreatment with the PKC activator PDBu (100 nM), but not with the inactive 4 α -PDD (100 nM), abolished the ACH-induced Ca^{2+} -signal. Inhibition of PKC activation by Ro 32-0432, Gö 6976 or bisindolylmaleimide I significantly diminished the carbachol (3 μM)-induced Ca^{2+} -signal by about 40-50% ($p < 0.01$) demonstrating that receptor-mediated activation of PKC provides positive feedback on the generation of the carbachol-induced Ca^{2+} -signal. In Ca^{2+} -free medium carbachol (10 μM) caused a transient rise in $[\text{Ca}^{2+}]_i$ by $175 \pm 24 \text{ nM}$ ($n = 12$) reflecting IP_3 -dependent mobilization of internal Ca^{2+} . This was completely inhibited by pretreatment with PMA (100 nM) and was somewhat enhanced by the PKC inhibitors indicating that activation of PKC exerts negative feedback on carbachol-induced internal Ca^{2+} mobilization. Furthermore, PKC activators as well as PKC inhibitors inhibited both voltage-sensitive Ca^{2+} -influx evoked by repetitive pulses of high K^+ (45 mM) and capacitative Ca^{2+} influx through non-L-Type channels evoked by thapsigargin (2 μM). **Conclusions:** In mouse beta-cells activation of PKC plays an important role in the generation of ACH-induced Ca^{2+} -signals by providing complex positive- as well as negative-feedback. Supported by DFG grant Scho 466/2-1.

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Depolarization causes calcium-induced calcium release in insulinoma cells

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Background and Aims: Ryanodine receptors have been identified in pancreatic beta cells, but evidence of Ca^{2+} -induced Ca^{2+} release from the endoplasmic reticulum (ER) is lacking. We asked how a depolarization-induced rise in cytoplasmic Ca^{2+} would affect ER Ca^{2+} , and whether drugs that inhibit or activate the ryanodine receptor could alter the response.

Materials and Methods: We verified the presence of ryanodine receptors in MIN6 cells (glucose-responsive mouse insulinoma cell line) by immunocytochemistry and by BODIPY-FL-X-ryanodine binding studies. The cells were transfected with ER-targeted 'cameleon' Ca^{2+} indicators, which report changes in ER Ca^{2+} through changes in fluorescence resonance energy transfer between pairs of green fluorescent proteins. Under illumination at 440 nm, 535/485nm emission ratio, which reflects the concentration of Ca^{2+} , was detected in individual live cells, and the effects of KCl, caffeine, and ryanodine were studied. We also examined cytoplasmic Ca^{2+} in cells loaded with the Ca^{2+} -sensing ratiometric dye Fura2-FF.

Results: With the baseline 535/485nm arbitrarily set at 1, depolarization with 25 mM KCl caused a drop to 0.90 ± 0.003 ($P < 0.0001$). This drop in ER Ca^{2+} was reversible with diltiazem which blocks L-type voltage-gated Ca^{2+} channels. Caffeine (10 mM) mimicked the effect of KCl causing a drop in the ratio to 0.85 ± 0.006 . However, there was no significant drop in the 535/485nm ratio in cells treated with 10 mM ryanodine (post-KCl ratio of 0.98 ± 0.012). The post-KCl 535/485nm ratio was significantly higher in cells treated with ryanodine than in those treated with vehicle ($P < 0.0001$), indicating a blockade of ER Ca^{2+} release. In the Fura-loaded cells, KCl caused an increase in the 340/380nm ratio from 0.26 ± 0.004 to 0.38 ± 0.002 in cells treated with vehicle, vs. an increase from 0.27 ± 0.008 to 0.32 ± 0.012 in ryanodine-treated cells ($P = 0.009$).

Conclusions: We have demonstrated that the ER can release Ca^{2+} in response to a rapid increase in cytoplasmic Ca^{2+} due to depolarization of pancreatic beta cells. Our data suggest that Ca^{2+} -induced Ca^{2+} release through the ryanodine receptor contributes to the cytoplasmic Ca^{2+} signal generated by activation of voltage-gated Ca^{2+} channels.

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CALCIUM TRANSIENTS IN PANCREATIC BETA CELLS ARE TRIGGERED BY INHIBITORS OF CALCINEURIN AND SUPPRESSED BY LEPTIN

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Background and Aims: Rise of cyclic AMP promotes IP_3 -mediated generation of transients of cytoplasmic Ca^{2+} assumed to be involved in the synchronization of the β -cell activity in the pancreas. The aim of the study was to examine how calcineurin-induced dephosphorylation and leptin affect the firing of these transients. **Methods:** Single cells and small aggregates were prepared from *ob/ob*, *ob/+* and mice lacking the *ob* gene. The cytoplasmic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) was measured with a digital imaging technique. Blockade of the voltage-dependent Ca^{2+} channels with methoxyverapamil made it possible to examine $[\text{Ca}^{2+}]_i$ transients due to intracellular release without background of periodic Ca^{2+} influx. **Results:** Glucose-induced transients of $[\text{Ca}^{2+}]_i$ often appeared in synchrony with those in other β -cells separated by distances up to 80 μm . The frequency of the transients was very low ($< 0.01/\text{min}$), but increased dramatically when raising the cyclic AMP content with glucagon, forskolin, theophylline or caffeine. The β -cells from the *ob/ob* mice were more active in generating $[\text{Ca}^{2+}]_i$ transients than those from the lean mice, resulting in frequencies of 0.2–0.5/min in the presence of 20 nmol/l glucagon. The calcineurin inhibitors cyclosporin A and FK506 increased the number of $[\text{Ca}^{2+}]_i$ transients in β -cells from *ob/ob* mice by 23% ($P < 0.05$) and 43% ($P < 0.001$) respectively when added at concentrations of 10 $\mu\text{mol/l}$ to a glucagon-containing medium. Leptin (1 and 10 nmol/l) reduced the number of transients by 15% ($P < 0.025$) and 23% ($P < 0.01$) respectively. **Conclusions:** Both cyclic AMP phosphorylation of the IP_3 receptor and its dephosphorylation by calcineurin appear to modify the generation of $[\text{Ca}^{2+}]_i$ transients. Pancreatic β -cells from *ob/ob* mice are characterized by excessive firing of transients, probably due to the absence of leptin.

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FINELY TUNED REGULATION OF STORE-OPERATED Ca^{2+} INFLUX INTO MOUSE PANCREATIC β -CELLS

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Background and aims: A store-operated mechanism for depolarising entry of cations has been proposed to be a determinant of the electrical activity underlying insulin secretion. The regulation of store-operated Ca^{2+} entry was therefore studied in individual mouse pancreatic β -cells.

Materials and Methods: The cytoplasmic concentrations of Ca^{2+} ($[\text{Ca}^{2+}]_i$) and Mn^{2+} ($[\text{Mn}^{2+}]_i$) were measured with the fluorescent indicator fura-2. Influx through the store-operated pathway was initially shut off by preexposure to 20 mM glucose, which maximally stimulates intracellular Ca^{2+} sequestration. To avoid interference with voltage-dependent Ca^{2+} entry the β -cells were hyperpolarized with diazoxide and the channel blocker methoxyverapamil was also present. Activation of the store-operated pathway in response to Ca^{2+} depletion of the endoplasmic reticulum was estimated from the sustained elevation of $[\text{Ca}^{2+}]_i$ or from the rate of increase in $[\text{Mn}^{2+}]_i$ due to influx of these extracellular ions.

Results: Increasing concentrations of the inositol 1,4,5-trisphosphate-generating agonist carbachol or the sarco(endo)plasmic reticulum Ca^{2+} -ATPase inhibitor cyclopiazonic acid (CPA) caused gradual activation of the store-operated pathway. Also the carbachol- and CPA-induced influx of Mn^{2+} depended on store filling in a graded manner. The store-operated influx of $\text{Ca}^{2+}/\text{Mn}^{2+}$ was inhibited by Gd^{3+} and 2-aminoethoxydiphenyl borate, but neither of these agents discriminated between store-operated and voltage-dependent entry.

Conclusions: The store-operated Ca^{2+} influx into β -cells is regulated by Ca^{2+} -store filling in a graded rather than an all-or-non-fashion. The depolarising influence of the store-operated current may be a determinant for the characteristic electrophysiological bursting pattern.

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MEMBRANE STRETCH IS A TRIGGER OF Ca^{2+} TRANSIENTS IN PANCREATIC β -CELLS BY MOBILIZING INTRACELLULAR Ca^{2+} STORES

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Background and aims. Nonadrenergic, noncholinergic (NANC) neurons have been proposed to synchronize the islets in the pancreas by triggering β -cell transients of cytoplasmic Ca^{2+} via an IP_3 -dependent mechanism. It was tested whether pancreatic β -cell respond to stretch activation with similar types of Ca^{2+} signals and if these propagate to other β -cells in the presence and absence of cell contacts. **Methods.** Single cells and small aggregates were prepared from β -cell-rich islets from ob/ob-mice. The cells were cultured and the cytoplasmic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) was measured with digital imaging technique in the presence of 20 mmol/l glucose and 50 $\mu\text{mol/l}$ methoxyverapamil. Membrane stretch was induced by osmotic swelling or focal touch stimulation. **Results.** Lowering the external medium osmolarity with 100 mosmol/l by removal of sucrose or by medium dilution resulted in 2-3 fold increase in the number of transients, reaching frequencies of 0.10-0.15/min during an initial 5 min period. In support for the idea that the transients were generated by volume expansion, sucrose omission was stimulatory also after isoosmolar replacement with readily penetrating urea. The intracellular Ca^{2+} -ATPase inhibitor thapsigargin suppressed both the spontaneously occurring transients and those activated by volume expansion. Touch stimuli induced $[\text{Ca}^{2+}]_i$ transients, which rapidly propagated to cells occurring within the same aggregate or lacking contact. The ability to react to extracellular signals generated by touch diminished with the distance to the stimulated cell, leaving the amplitudes of the transients unaffected. **Conclusions.** Membrane stretch triggers $[\text{Ca}^{2+}]_i$ transients of intracellular origin similar to those proposed to coordinate the rhythmicity of the islets in the pancreas. Touch stimulation is a useful tool for investigating the propagation of $[\text{Ca}^{2+}]_i$ signals between pancreatic β -cells lacking contact.

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EXPRESSION AND FUNCTION OF THE EXTRACELLULAR CALCIUM-SENSING RECEPTOR IN MIN6 CELLS

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Background and Aims: The extracellular calcium-sensing receptor (CaR) is the mechanism through which cells involved in the systemic regulation of Ca^{2+} recognise and respond to changes in extracellular Ca^{2+} . We have recently demonstrated that β -cells in human islets of Langerhans express the CaR. We have now investigated whether the mouse insulinoma MIN6 line also expresses the CaR, and whether CaR activation influences MIN6 cell function. **Materials and Methods:** Expression of CaR mRNA by MIN6 cells and primary mouse islets was determined by RT-PCR, changes in $[\text{Ca}^{2+}]_i$ were assessed by microfluorimetry and insulin secretion from perfused MIN6 cells was measured by radioimmunoassay. **Results:** MIN6 cells and mouse islets expressed a mRNA species which amplified using PCR primers specific for the mouse parathyroid CaR, giving a single product of the predicted size, identical to that amplified in parallel from mouse kidney cDNA. Single cell Ca^{2+} microfluorimetry demonstrated that increasing extracellular Ca^{2+} to activate the CaR (0.5mM to 10mM Ca^{2+} , 2mM glucose) produced elevations in $[\text{Ca}^{2+}]_i$ in 76% of MIN6 cells in 3 experiments. CaR activation also had marked effects on insulin secretion from perfused MIN6 cells configured as pseudoislets. Increasing extracellular Ca^{2+} from 0.5 to 10mM caused a marked, prolonged and fully-reversible inhibition of insulin secretion in the presence of both substimulatory glucose (2mM, $27 \pm 5\%$ control, $n=3$) or stimulatory glucose (20mM, 50% control). **Conclusions:** MIN6 cells express the CaR and its activation has effects that were similar to those observed in human primary islets. These observations suggest a common function for the β -cell CaR, and confirm that MIN6 cells offer a useful experimental model in which to further study the regulation of β -cell function by CaR activation.

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INVOLVEMENT OF A STORE-OPERATED MECHANISM IN GLUCOSE INHIBITION OF GLUCAGON SECRETION

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Background and aims: Little is known about the mechanism underlying glucose inhibition of glucagon secretion. We now study the effect of glucose on the cytoplasmic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$), which controls glucagon secretion.

Materials and Methods: Single cells were prepared from pancreatic islets of normal mice.

$[\text{Ca}^{2+}]_i$ was measured with a digital imaging technique and the cells were then identified by immunostaining.

Results: About 5% of the α -cells exhibit spontaneous activity with large amplitude oscillations of $[\text{Ca}^{2+}]_i$ in the 0.2-0.5 /min range in the presence of 3 mM glucose. Most α -cells react to 5 $\mu\text{mol/l}$ adrenaline, 2-3 mmol/l glutamine or glycine with oscillations or sustained elevation of $[\text{Ca}^{2+}]_i$ due to influx through voltage-dependent channels. However, the adrenaline response involves also initial mobilisation of intracellular Ca^{2+} . Depending on stimulus, rise of the glucose concentration to 20 mM causes partial or complete inhibition of the $[\text{Ca}^{2+}]_i$ response. Glucose also stimulates intracellular Ca^{2+} sequestration into the pool mobilised by adrenaline. Inhibitors of the sarco(endo)plasmic reticulum Ca^{2+} ATPase mimicks the stimulatory effects of adrenaline causing oscillations or sustained elevation of $[\text{Ca}^{2+}]_i$, which are inhibited by hyperpolarisation or by blockers of voltage-dependent Ca^{2+} channels.

Conclusions: Release of intracellular Ca^{2+} causes depolarisation by activation of a store-operated current in the α -cell. This effect alone is sufficient for activation of a more prominent voltage-dependent entry of the ion. By stimulating Ca^{2+} sequestration in the ER, glucose can inactivate the store-operated current. We propose that this effect may contribute to or explain glucose inhibition of glucagon secretion.

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EVIDENCE THAT ATP PRODUCTION OF B-CELL MITOCHONDRIA AND K^+_{ATP} CHANNEL INHIBITION ARE LINKED BY CREATINE PHOSPHATE

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Background and Aims: It has been shown that the ATP which closes K^+_{ATP} channels in the stimulus-secretion coupling (SSC) of pancreatic B-cells exclusively derives from cytosolic NADH production during glycolysis (cNADH). It is yet unclear how the cells can discriminate between reduction equivalents generated in the cytosol or in the citrate cycle. We propose that ATP derived from cNADH may be specifically transformed into another K^+_{ATP} channel mediator.

Materials and Methods: Creatine phosphate (CrP_i) was tested for effects on K^+_{ATP} currents in inside/out-patches of native B-cells and on currents through B-cell K^+_{ATP} channels expressed in *Xenopus* oocytes. Effects of creatine kinase (CrK) inhibitors were tested on cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) by fura-2 fluorescence and on L-type Ca^{2+} channel currents in B-cells.

Results: K^+_{ATP} currents in oocytes appeared after poisoning the cells with 5 mM NaN_3 . Subsequent injection of 50 nl of a 100 mM ATP or CrP_i solution diminished the current at +40 mV from 3.11 ± 0.50 μA to 1.20 ± 0.15 μA ($n=10$) and from 3.40 ± 0.59 μA to 1.18 ± 0.16 μA ($n=14$), respectively. Injection of creatine ($n=9$) or phosphate ($n=4$) (100 mM each) was without effect. CrP_i (1mM) alone had no effect on single channel K^+_{ATP} currents ($n=5$), but reduced P_o in the presence of ADP and ATP (500 μM each) by $85 \pm 4\%$ ($n=17$). This points to the involvement of a CrK in this process. Consequently, substances known as inhibitors of CrK (fluorodinitrobenzene, 30 μM ; iodoacetamide, 25 μM) abolished glucose-induced elevations in $[Ca^{2+}]_i$ ($n=4$ and 3, respectively), while having only slight effects on L-type Ca^{2+} channel currents at 100 μM or 1 mM ($n=5$ and 6, respectively).

Conclusions: Energy rich phosphate may be shuttled as CrP_i between mitochondria and the plasma membrane. The cells seem to transform mitochondrial ATP from cNADH to cytosolic CrP_i which is converted back into ATP close by the K^+_{ATP} channel.

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Effects Of Leptin On Insulin Secretion In Rat Islets In Vitro

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Background and Aims: In recent studies there are some controversial results about effects of leptin on insulin secretion in islets. We speculated that the reason might be the differences of those experiments' designs. Our aim was to investigate the effects of leptin of various concentrations on insulin secretion in rat islets in vitro.

Material and Methods: Islets were isolated from SD rats and incubated in the absence or presence of recombinant rat leptin (R&D, 0, 1, 5, 10, 15, 50 or 100ng/ml) at either 5.6mM or 16.7mM glucose for 10 min or 2h. After incubation, the medium was removed and assayed for insulin by radioimmunoassay.

Results: At 5.6 mM glucose, a 10-min incubation with 1ng/ml or 5ng/ml leptin stimulated insulin secretion (26.47 ± 2.47 and 26.54 ± 1.79 mU/L, respectively, $P < 0.05$) as compared to 0ng/ml leptin (24.32 ± 1.28 mU/L), and a 2h incubation with 5ng/ml leptin also stimulated insulin secretion (31.74 ± 2.37 mU/L, $P < 0.05$), but a 2h incubation with ≥ 50 ng/ml leptin inhibited insulin secretion (23.88 ± 2.95 and 23.56 ± 3.43 mU/L, respectively, $P < 0.05$) as compared to 0ng/ml leptin (27.58 ± 2.53 mU/L). At 16.7 mM glucose, a 10-min incubation with ≥ 50 ng/ml leptin inhibited insulin secretion (23.73 ± 1.40 and 23.69 ± 1.34 mU/L, respectively, $P < 0.05$) as compared to 0ng/ml leptin (8.29 ± 2.53 mU/L), and a 2h incubation with ≥ 5 ng/ml leptin also inhibited insulin secretion (31.19 ± 4.42 , 27.44 ± 1.83 , 27.20 ± 3.79 , 26.37 ± 2.43 and 24.37 ± 0.57 mU/L, respectively, $P < 0.05$) as compared to 0ng/ml leptin (35.06 ± 4.33 mU/L).

Conclusions: Recombinant leptin has a biphasic effect on insulin secretion in rat islets in vitro, which can be influenced by several factors including leptin concentration, circumstance glucose concentration and exposure time.

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Diazoxide inhibits ATP production in isolated β -cell mitochondria only in the presence of extramitochondrial Mg-nucleotides

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Background and Aims: Diazoxide (DZ) binds to the sulphonylurea receptor (SUR) of ATP regulated potassium channels in the plasma membrane and prevents β -cell depolarization and insulin release. In addition, DZ impairs β -cell respiration and mitochondrial Ca^{2+} handling. Mitochondrial SUR's have been identified in cardiac myocytes and in hepatocytes thus mitochondrial effects in β -cells might be mediated by DZ binding to mitochondrial SUR as well. This study aims at characterizing the effect of DZ on ATP production in isolated β -cell mitochondria.

Materials and Methods: Isolated β -cell mitochondria were incubated for 10 min at 37 °C with various substrates in the presence of DZ. Mitochondrial ATP production (MAPR) induced by oxidative phosphorylation was normalized by ATP production induced by mitochondrial adenylate kinase detected in the absence of substrates. ATP was detected with a bioluminometric method.

Results: DZ (10-1000 μM) had no effect on MAPR induced by pyruvate/malate (1mM/1mM; 4.59 ± 0.41 in the absence vs. 4.26 ± 0.66 in the presence of 1000 μM DZ), α -ketoisocaproate/glutamate (0.1mM/10mM; 2.45 ± 0.66 vs. 2.62 ± 0.35) or glycerol 3-phosphate (10mM; 1.55 ± 0.16 vs. 1.46 ± 0.23) excluding a direct but unspecific inhibition of the respiratory chain. Addition of MgATP (2 μM) rendered MAPR induced by pyruvate/malate sensitive towards DZ. A concentration-dependent inhibition was observed at 100 μM DZ (10%, $p > 0.05$ vs ATP free control), 300 μM DZ (20%, $p < 0.05$) or 1000 μM DZ (25%, $p < 0.05$). Similar results were obtained with MgCTP (1 mM).

Conclusions: DZ inhibits ATP production in isolated β -cell mitochondria only in the presence of (cytoplasmic) Mg-nucleotides. The requirement of MgATP is characteristic for DZ binding to SUR indicating the presence of a functional active SUR in the inner membrane of β -cell mitochondria.

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LEPTIN INHIBITS GLUCOSE-STIMULATED INSULIN SECRETION WITHOUT DECREASING ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE LEVELS IN ISLETS

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Background and Aims: It is debated whether leptin inhibits glucose-induced insulin secretion and which its mechanism of action might be. It has been recently claimed that leptin decreases cellular cAMP levels through the activation of the cyclic nucleotide phosphodiesterase 3B.

Materials and Methods: Therefore, we have studied the effect of mouse-recombinant leptin on the insulin secretory response (ng/30 min \times μg DNA) of rat perfused islets to glucose. Moreover, the effect of leptin on glucose utilization ($3H_2O$ -production from D-[5-3H]glucose), glucose oxidation ($^{14}CO_2$ -production from D-[U- ^{14}C]glucose) and islet cAMP levels (fmol/ μg DNA; ELISA method) was also investigated.

Results: The biphasic insulin response to 20 mmol/l glucose was significantly suppressed by 5 nmol/l (33 ± 3 , $n=8$, vs. 90 ± 4 , $n=8$; $p < 0.001$) but not 1 nmol/l leptin. The inhibitory effect of leptin was completely blocked in the presence of either 250 $\mu mol/l$ 8-bromo-cAMP (103 ± 7 , $n=9$, vs. 125 ± 11 , $n=8$; N.S.) or 100 nmol/l trequinsin (a specific inhibitor of the cyclic nucleotide phosphodiesterase 3B) (134 ± 8 , $n=10$, vs. 120 ± 10 , $n=10$; N.S.). However, 166 nmol/l wortmannin (a phosphatidylinositol 3-kinase inhibitor) did not modify the glucose response in the presence of leptin (36 ± 3 , $n=10$, vs. 39 ± 3 , $n=10$; N.S.). A change of the glucose concentration from 3 to 20 mmol/l increased islet cAMP significantly (1212 ± 99 , $n=9$, vs. 531 ± 31 , $n=9$; $p < 0.001$). This stimulation was not decreased by leptin (1289 ± 101 , $n=9$), neither increased by trequinsin (1141 ± 116 , $n=9$), but it was significantly enhanced by the combination of both leptin and trequinsin (1785 ± 119 , $n=9$, vs. 1212 ± 99 , $n=9$; $p < 0.002$). Leptin modified neither glucose utilization nor glucose oxidation at either 3 or 20 mmol/l glucose.

Conclusions: Mouse recombinant leptin inhibits glucose-induced insulin secretion without impairing glucose-induced increase of cAMP levels. Therefore, leptin-induced suppression of insulin secretion is not due to a decreased generation of cAMP. The observed synergism between leptin and trequinsin on islet cAMP levels speaks against an activation of the cyclic nucleotide phosphodiesterase 3B by leptin.

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CALCIUM SIGNALLING VIA L-TYPE CALCIUM CHANNELS MEDIATES IL-1-INDUCED ACTIVATION OF C-JUN N-TERMINAL KINASE AND p38 IN PANCREATIC BETA-CELLS

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Background and Aims: Interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF α) and interferon- γ (IFN γ) have been proposed as immune effectors of pancreatic beta-cell death in type 1 diabetes mellitus. Activation of c-jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinases (MAPKs) and increased calcium signalling through T- and L-type calcium channels are essential signalling elements in the beta-cell apoptotic pathway induced by IL-1 β or a combination of cytokines. We previously found that inhibition of T-type calcium channels does not affect cytokine-induced MAPK activity. In this study we explored the putative involvement of L-type channel-mediated calcium signalling in regulating IL-1 β -induced MAPK activation in beta-cells.

Materials and Methods: JNK, ERK and p38 activities in whole cell lysates from isolated ob/ob mouse islets or mouse beta-TC3 cells were determined in a whole cell lysate phosphotransferase assay using c-jun, Elk-1 and heat shock protein 25 (Hsp25) as substrates.

Results: Exposure of ob/ob islets to 400 U/ml of IL-1 β induced a 2.3-fold ($p < 0.0005$) increase in c-jun phosphorylation and a 1.4-fold ($p < 0.0005$) increase in Hsp25 phosphorylation, reflecting increased activities of JNK and p38, respectively. No increase in Elk-1 phosphorylation was observed, indicating that ERK was not activated by IL-1 β . Combined inhibition of T- and L-type calcium channels using mibefradil and nimodipine reduced IL-1 β -stimulated JNK activity to 1.8-fold ($p < 0.01$) and completely prevented ($p < 0.001$) p38 activation by IL-1 β . Similar results were obtained with beta-TC3 cells.

Conclusions: These results provide evidence that IL-1 β signal transduction leading to JNK and p38 activation is dependent on calcium signalling through L-type calcium channels in beta-cells.

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MODULATION OF ISLET ISOFORMS OF NITRIC OXIDE SYNTHASE BY DIFFERENT INSULIN SECRETAGOGUES

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Aims: Nitric oxide synthase (NOS) has been detected in the islets of Langerhans and NO has been implicated as a negative feedback regulator of glucose-stimulated insulin release. We recently found that high concentrations of glucose stimulated the activities of both constitutive NOS (cNOS) and inducible NOS (iNOS) in incubated islets. The present study was performed to elucidate, whether non-glucose insulin secretagogues might influence islet NOS activities in relation to insulin release.

Methods: Isolated mouse islets were incubated in the presence of different insulin secretagogues; i.e. glucose, L- and D-arginine, L-leucine, α -ketoisocaproic acid (KIC) and carbachol. Insulin release is assayed with RIA and islet activities of cNOS and iNOS with a sensitive HPLC-method. Results. Western blot revealed that the islets incubated in a basal, non-insulin stimulating glucose concentration contained cNOS but not iNOS protein, whereas high glucose increased both proteins. L-arginine stimulated islet cNOS activity but did not affect islet iNOS activity. D-arginine displayed a slight cNOS stimulating effect. Insulin release stimulated by glucose or L-arginine was potentiated by addition of a NOS-inhibitor. L-leucine and KIC stimulated insulin release without affecting either islet cNOS- or iNOS-activity. Similar to L-arginine, carbachol stimulated islet cNOS but induced in addition a very slight iNOS activity. Carbachol-stimulated insulin release was potentiated by NOS-inhibition.

Conclusion: The present data suggest that glucose, among the tested secretagogues, is a major inducer of islet iNOS activity and that the mitochondrial substrates KIC and L-leucine exert their action downstream of glucose-stimulated iNOS induction in the metabolic transduction pathway. The effect of L-arginine and carbachol on cNOS activity is probably exerted by their action to stimulate an increase in intracellular Ca $^{2+}$, being stimulatory to the Ca $^{2+}$ /calmodulin dependent cNOS activity.

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PROTEIN INHIBITOR OF NEURONAL NITRIC OXIDE SYNTHASE IS EXPRESSED IN RAT B-CELLS AND MODULATES INSULIN SECRETION.

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Background and Aims: In our previous molecular studies, we showed the expression of a neuronal isoform of nitric oxide synthase (nNOS) that controls insulin secretion in rat pancreatic b-cells. PIN (Protein Inhibitor of Neuronal NOS) is an endogenous inhibitor of nNOS previously identified in rat brain. Our work was aimed at investigating 1) the presence of PIN in b-cells, 2) its subcellular localization and 3) its role in the insulin secretion.

Materials and Methods: Molecular and biochemical experiments were applied to isolated rat islets and to the insulin-secreting cell line INS-1. For functional studies, PIN was overexpressed in INS-1 cells by transfection of an expression vector containing PIN and insulin secretion was measured after incubation with Krebs Ringer bicarbonate buffer containing 5 mM glucose during one hour.

Results: Using RT-PCR, we showed the expression of PIN mRNA in islets and in INS-1 cells. The presence of a protein of 10 kDa identical to the brain PIN is demonstrated by Western blot. Immunofluorescence studies performed in INS-1 cells with a PIN antibody showed a strong colocalization with the neuronal NOS staining. This was confirmed by electron microscopy which evidences the presence of PIN in the insulin granules as nNOS and to a lesser extent in the cytoplasm of the b-cells. Furthermore, overexpression of PIN in INS-1 cells (5 fold over an empty vector) induced a 75% increase of the insulin secretion induced by 5 mM glucose as compared to control cells.

Conclusions: PIN, the endogenous inhibitor of nNOS, is expressed in rat b-cells, is strongly colocalized with nNOS and appears to be a positive modulator of insulin secretion.

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SECRETORY PHOSPHOLIPASE A $_2$ IS RELEASED FROM PANCREATIC β -CELLS AND PROMOTES INSULIN RELEASE BY K $_{ATP}$ -CHANNEL CLOSURE
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Aim: To measure the release of secretory phospholipase A $_2$ (sPLA $_2$) from pancreatic β -cells and evaluate its role in modulation of insulin secretion.

Methods: A sPLA $_2$ fluorescent substrate, Bis-BODIPY FL phosphatidylcholine, loaded into the outer leaflet of the plasma membrane was used to monitor the release of sPLA $_2$ from single mouse β -cells. ATP-sensitive K $^+$ (K $_{ATP}$)-channel activity and exocytosis were recorded using patch-clamp techniques. Cytoplasmic free Ca $^{2+}$ levels ([Ca $^{2+}$] $_i$) were determined by microfluorometry. Insulin was assayed by ELISA. **Results:** Extracellular application of sPLA $_2$ (0.1-5 μ M) stimulated insulin secretion from intact mouse islets at 3 mM (>300 %) and 16.7 mM (>270 %) glucose. The insulinotropic action of sPLA $_2$ was secondary to closure of K $_{ATP}$ -channels: the whole-cell K $_{ATP}$ -conductance decreased from 0.62 \pm 0.20 to 0.18 \pm 0.04 nS/pF in the presence of sPLA $_2$ ($p < 0.01$). Inhibition of K $_{ATP}$ -channel activity by sPLA $_2$ was associated with gradual and irreversible increase in [Ca $^{2+}$] $_i$, that was mimicked with lyso-phosphatidylcholine (lyso-PC). In contrast, arachidonic acid (the other product of phospholipid hydrolysis catalysed by sPLA $_2$) elicited a fast and reversible increase in [Ca $^{2+}$] $_i$. Mixture of glucose and KCl induced an increase in BODIPY FL fluorescence due to release of sPLA $_2$ from β -cells with consequent breakdown of the fluorescent substrate by the released enzyme. Secretory PLA $_2$, as well as arachidonic acid and lyso-PC, did not affect Ca $^{2+}$ -dependent exocytosis, measured as increases in cell capacitance. **Conclusion:** These data suggest that sPLA $_2$ is released from mouse β -cells in response to cell stimulation and the released phospholipase A $_2$ might play a role as a positive feedback signal to further enhance β -cell exocytosis by inhibition of K $_{ATP}$ -channel activity.

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STIMULUS-RESPONSE COUPLING IN MIN6 CELLS OVER- AND UNDER-EXPRESSING CYTOSOLIC PHOSPHOLIPASE A₂

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Background and Aims: Cytosolic phospholipase A₂ (cPLA₂) has been implicated in coupling increases in intracellular Ca²⁺ ([Ca²⁺]_i) to the release of insulin. We have now investigated the effects of over- and under-expression of cPLA₂ on β-cell function. **Materials and Methods:** MIN6 cells were stably transfected with sense and antisense cPLA₂ vectors. cPLA₂ expression was determined by Western blotting, changes in [Ca²⁺]_i were assessed by microfluorimetry, insulin secretion from perfused MIN6 cells was measured by radioimmunoassay and preproinsulin (PPI) mRNA levels were determined by real-time PCR. **Results:** Western blotting of stably transfected cell lines confirmed over- and under-expression of cPLA₂. cPLA₂ overexpression was without significant effect on 100μM tolbutamide- or 20mM KCl-induced increases in [Ca²⁺]_i (P>0.2). However, the responsiveness to nutrients was dramatically reduced, with only 4% of cells showing a Ca²⁺ response to 20mM glucose and 8% responding to 10mM KIC, compared with 61% and 94% of control cells responding (n=28-52 cells, P<0.001). Inhibition of cPLA₂ activity with 50μM methyl arachidonate fluorophosphonate partially restored glucose-induced elevations in Ca²⁺ (P<0.01). cPLA₂ overexpressing cells were also insensitive to 20mM glucose in terms of insulin secretion (sense: 109±15.2% basal; controls: 326±24.9% basal, n=3, P<0.01). Underexpression of cPLA₂ had minimal effects on increases in [Ca²⁺]_i in response to nutrients and depolarising stimuli (P>0.2 versus controls), and it did not affect the profile or magnitude of initial insulin secretory responses. However, cPLA₂-deficient cells were unable to sustain a secretory response upon prolonged stimulation. Insulin content was reduced by 90% following underexpression of cPLA₂ (P<0.001), but PPI mRNA levels were not significantly different (control: 16.6±2.8fg; antisense: 14.0±2.7fg, P>0.2). **Conclusions:** These data suggest that overexpression of cPLA₂ inhibits glucose handling by β-cells without affecting downstream signalling events, and that normal expression of cPLA₂ may be required for maintaining insulin stores.

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Receptor-Mediated Signalling in Beta-Cells

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CULTURE OF RAT BETA-CELLS WITH INCRETIN HORMONES PRESERVES THEIR SUBSEQUENT SECRETION RESPONSIVENESS

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Background and Aims: We recently observed that 2-24 h exposure of rat beta-cells to low (3 mmol/l) glucose, suppresses their subsequent capacity to release insulin. In the present study we examined to which extent addition of the gluco-incretin hormones GLP-1 (glucagon-like peptide [7-37 amide]) and/or GIP (glucose-dependent insulinotropic peptide) could protect against this secretory failure.

Materials and Methods: FACS-purified rat beta-cells were cultured for 24 h in control medium with alternating glucose concentration of 10 mmol/l (G10: h 0-1, h 7-8 and h 17-18 of culture) and 3 mmol/l (G3: h 1-7, h 8-17 and h 18-24 of culture) as well as in test media in which the G10 condition was supplemented with GLP-1 and/or GIP. After culture, the cells were perfused at 3 mmol/l glucose and stimulated during 10 min pulses at G10 and at G10 with 10 nmol/l GLP-1. Insulin release was measured during culture and during perfusion.

Results: Insulin release induced by G10 in beta-cells exposed for 6 h to G3 was decreased by 88±7% as compared to the prior G10 stimulation (0.08±0.04 vs 0.65±0.04 ng.10³ cells⁻¹.h⁻¹; P<0.001). Further incubation in G3 did not enhance the secretory defect. The loss in secretory response was limited to 50±8 % by addition of 1 nmol/l GLP-1 to the G10 medium; even better protection was observed by addition of a mixture of GLP-1 and GIP (total concentration = 1 nmol/l) to the G10 medium. Addition of these peptides stimulated cAMP accumulation in the cultured cells but did not alter their insulin content. The beta-cells which had been cultured in the presence of GLP-1/GIP exhibited a stronger secretory response during subsequent perfusion at 10 mmol/l glucose (release of 0.060±0.007 % of insulin content vs 0.022±0.003 % in control preparation; P<0.05). This was also the case when perfused at 10 mmol/l glucose plus GLP-1 (release of 0.86±0.08 % of insulin content vs 0.41±0.06 % in control preparation; P<0.05).

Conclusions: In a model of alternating exposure of beta-cells to 3 and 10 mmol/l glucose, supplementing G10 culture medium with incretins better preserves the insulin releasing capacity of the cells than observed for cells cultured with glucose alone.

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Establishment of a 4-OH-Tamoxifen-inducible CaM-Kinase II-MER fusion-protein

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Background and Aims: Ca²⁺/calmodulin dependent protein kinase II (CaMK II) is highly expressed in beta-cells and is thought to be involved in insulin-secretion and insulin gene-expression. Previous reports showed activation of MAP kinases via CaMK II and phosphorylation of CREB by constitutively active mutants of CaMK II. This raised the question whether CaMK II modulates gene expression at unclear sites in beta-cells. The aim of this work was to establish a 4-OH-Tamoxifen inducible CaMKII-construct by cloning the kinase domain (aa 1-293) of the enzyme in front of a mutated estrogen receptor (MER) and to explore whether this fusion-protein is functional and able to activate MAP-kinase and CREB in an inducible manner. The mutated estrogen receptor contains no nuclear-localisation-signal (NLS). The binding of heat shock proteins inhibits the kinase-activity until 4-OH-Tamoxifen displaces them and thereby allows a rapid activation of kinase-activity.

Materials and Methods: The kinase-domain of CaMK II delta2 was amplified by PCR and cloned into a pcDNA3.1/His B-vector (Invitrogen) containing the mutated estrogen receptor (kindly provided by Prof. Dr. U. R. Rapp, Wuerzburg, Germany). The resulting construct was transiently transfected into HEK293 cells by the calcium phosphate method. The activity of the expressed kinase was determined by radioactive kinase-activity-assays. The activation of MAP-kinase, CREB and ATF-2 was measured by use of the PathDetect System (Stratagene) and Dual Luciferase reporter-gene-assay (Promega).

Results: The pcDNA3.1/His B CaMK II MER-construct was found to be highly inducible by 200 nmol/l 4-OH-Tamoxifen. Kinase-activity-assays revealed a 15 (p=0.001) times higher activity in Ca²⁺ free buffer belonging to the Ca²⁺ independent activity of the CaMK II kinase-domain. Cotransfection of pcDNA3.1/His B CaMK II MER and either CREB, Elk-1 (as a marker for MAP-kinase activity) or ATF-2 (the pathway which leads to activation of ATF-2 is not characterized yet) did not lead to an activation of CREB, MAP-kinase or ATF-2, respectively. In a control PKA caused a strong activation of the cotransfected CREB demonstrating the correct function of the PathDetect system.

Conclusions: A highly 4-OH-Tamoxifen-inducible fusion-protein of the CaMK II kinase-domain and the mutated estrogen receptor could be established as determined by kinase-activity assays. The fusion-protein was not able to activate neither the MAP-kinase cascade represented by Elk-1 nor the cAMP pathway represented by CREB nor ATF-2 leading to the conclusion that CaMK II does not directly phosphorylate nuclear proteins to alter gene transcription.

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SELECTIVE SIGNALING VIA A- AND B-TYPE INSULIN RECEPTORS IN PANCREATIC B-CELLS INVOLVES DIFFERENT PI3K

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Background and Aims: Pancreatic B-cells are targets for positive feedback action by secreted insulin. We have shown that secreted insulin activates the transcription of its own gene by signaling through insulin receptor (IR) A-type (Ex11-) and p70s6k while it activates transcription of the B-cell glucokinase gene (bGK) via IR B-type (Ex11+) and PKB(c-Akt). To start to understand the molecular mechanisms that underlie the selective signaling via the two IR isoforms we studied the PI3K involved.

Materials and Methods: As the readout for signaling via either IR-A or IR-B served insulin promoter-driven DsRed expression and bGK promoter-driven GFP expression, respectively, in co-transfected HIT-T15 and primary mouse B-cells. PI3K activity was measured in IR immunoprecipitates (IPs).

Results: 25 μM LY294002 or 50 nM Wortmannin in the culture medium blocked insulin-stimulated insulin promoter activity. It needed 100 μM LY294002 or 150 nM Wortmannin to block bGK promoter activity. PI3K activity in IR-A IPs was inhibited by 5-10 nM Wortmannin as described for PI3K classes I, II C2b and III. It needed 100 nM to block PI3K activity associated with IR-B, i.e. concentrations similar to those that inhibit PI3K class II C2a. Overexpression of the dominant-negative acting form of the class Ia adapter protein p85, i.e. Delta-p85, abolished insulin-stimulated insulin promoter activity but had no insulin-stimulated bGK promoter activity. Western blot analysis of IPs obtained with anti-PI3K C2a-antibodies revealed the presence of IR.

Conclusions: Our data suggest that one of the molecular mechanisms involved in the selective signaling via the two IR isoforms in the pancreatic B-cell is the selective activation of different classes of PI3K. Whereas activation of the insulin promoter via IR-A and p70s6k involves PI3K class Ia, it requires activation of a class II-like PI3K to activate bGK promoter via IR-B and PKB(c-Akt).

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ROLE OF LOCALLY RELEASED GLUCAGON IN THE INSULIN SECRETORY RESPONSE FROM THE INTACT PANCREAS

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Background and Aims: Glucagon is known as a potent stimulus of insulin release. It is recognized by glucagon- as well as by GLP-1- receptors (R) on the β -cells. Locally released glucagon uses both receptors to amplify glucose-induced insulin release in isolated islet preparations. The present study examines whether this is also the case in the intact pancreas.

Materials and Methods: Glucose-induced insulin release was measured in the perfused dorsal rat pancreas in the presence or absence of glucagon- or GLP-1 receptor-antagonists, namely and, respectively, [des-His1,des-Phe6,Glu9]-glucagon-NH₂ (desHG-GLU 10 μ mol/l) or exendin-(9-39)-NH₂ (exendin 1 μ mol/l). Receptor specificity and potency of both antagonists were determined by measuring cAMP production, phosphorylase activity and insulin release in isolated preparations of rat hepatocytes, transfected CHL-cells and rat β -cells.

Results: It was thus found that 1) desHG-GLU, but not exendin, was an effective antagonist of glucagon-induced cAMP production and phosphorylase activity in hepatocytes, which express glucagon-R but no GLP-1-R. 2) Exendin but not desHG-GLU, counteracted GLP-1- and glucagon-induced cAMP production in CHL-cells transfected with the GLP-1-R gene. 3) Both desHG-GLU and exendin counteracted glucagon-stimulated cAMP production and insulin secretion in purified β -cells. 4) None of the two antagonists exerted an inhibitory effect on the biphasic insulin release during pancreas perfusion at 20 mM glucose. On the other hand, they antagonized the stimulatory effect of their respective agonists, decreasing the potentiation of glucose-induced insulin release by exogenous glucagon by 82% ($p < 0.02$) or by exogenous GLP-1 by 90 % ($p < 0.02$). These antagonistic effects indicate that both compounds reach and interact with their cognate receptors during the perfusion experiment.

Conclusions: Under the present conditions of rat pancreas perfusion, glucose elicits a typical biphasic insulin release with an amplitude that appears not influenced by locally released glucagon.

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EXENDIN(9-39)AMIDE SUPPRESSES GLP-1 INSULINOTROPIC ACTION IN RATS INFUSED WITH DIMETHYL ESTER OF SUCCINIC ACID.

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Background and Aims: GLP-1 is a nutrient-dependent insulin secretagogue and markedly enhances insulin output from isolated perfused pancreas of normal and diabetic rats in the presence of dimethyl ester of succinic acid (SAD); also, iv injection of SAD potentiates the insulinotropic action of GLP-1 in normal and neonatally streptozotocin-treated rats. In this study we explored to which extent exendin(9-39)amide [Ex(9-39)], a known antagonist of GLP-1, may oppose the insulinotropic action of GLP-1 in rats infused with SAD. **Materials and Methods:** Anaesthetised fed male Wistar rats (241 \pm 8 g body wt) received, for 10 min, a primed constant infusion of SAD (0.5 μ mol/g followed by 0.25 μ mol/min, both per g body wt) and, at min 5, GLP-1 (0.5 pmol/g body wt) was injected iv. In control experiments, the same volume of saline was administered instead of SAD and/or GLP-1. In one set of SAD-infused rats, Ex(9-39) -a gift from Dr. J. Eng- was infused (5 pmol/min and per g body wt) from 1 min before to 3 min after GLP-1 injection. Blood samples (0.5 ml) were collected from a carotid artery for measurement of plasma glucose and insulin. Data are presented as mean \pm SEM. The statistical significance of difference between mean values was assessed by Student's t-test.

Results: In control experiments (n=4), plasma glucose and insulin concentrations were little affected, with mean paired difference (min 15 vs min zero) of -0.64 \pm 0.63 mM and -0.90 \pm 1.04 ng/ml, respectively. In SAD-infused rats, GLP-1 caused a progressive decrement in plasma glucose (2.32 \pm 0.37 mM at min 15, n=7, $p < 0.001$), which did not occur during Ex(9-39) infusion. SAD infusion provoked within 2 min a pair increment in plasma insulin (6.62 \pm 0.90 ng/ml, n=12, $p < 0.005$) which was maintained at min 5, whereas in the presence of Ex(9-39) it decreased (3.22 \pm 0.99 ng/ml, n=5, $p < 0.05$). GLP-1-induced integrated increment in insulin, over the first 5 min, was 11.93 \pm 2.73 (n=6), 40.16 \pm 6.60 (n=7) and 13.26 \pm 2.03 (n=5) ng x min/ml in saline-, SAD- and Ex(9-39)-infused rats, respectively. **Conclusions:** In SAD-infused rats, Ex(9-39) infusion decreased plasma insulin before GLP-1 injection, suppressed the B-cell response to GLP-1, and delayed and minimized its hypoglycemic action. It is proposed, therefore, that Ex(9-39) could represent a tool in the treatment of alimentary or reactive hypoglycemia.

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Evidence for regulation of glucagon release by protein tyrosine-phosphatases.

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Background and Aims: Augmented glucagon release may contribute to hyperglycaemia in diabetes, but the mechanism behind this phenomenon is unknown. Hence, we have investigated whether glucagon release from the pancreatic A-cells is regulated by protein tyrosine phosphatases (PTPases) in isolated islets of the Goto-Kakizaki (GK) rat, an animal model of hereditary type 2 diabetes.

Materials and Methods: Glucagon release was determined by radioimmunoassay in batch incubations of isolated GK and control Wistar (W) rat islets. To assess the role of PTPases in the regulation of glucagon secretion, a stable and selective inhibitor of PTPases, bisphosphonate-picolinate vanadate, or peroxovanadate (POV), was used.

Results: At 3.3 mmol/l glucose, basal glucagon release was unaffected by 1-10 μ mol/l POV, while higher concentrations of the compound, 0.1-1 mmol/l, enhanced glucagon release 5- and 12-fold (n=5-7; $p < 0.01$), respectively, from GK islets, and 5- and 25-fold (n=4-7; $p < 0.001$), respectively from W islets. Arginine (10 mmol/l) enhanced basal glucagon release 13.5-fold to 32.2 \pm 4.3 pg/islet per h in GK (n=8; $p < 0.001$) and 3.7-fold to 6.0 \pm 0.9 pg/islet per h (n=9) in W islets. POV (100 μ mol/l) inhibited arginine-induced glucagon release from GK islets (by 55%, n=8; $p < 0.05$) but did not significantly affect the release from W islets. At 1 mmol/l POV, arginine-stimulated glucagon release was unchanged in GK islets (32.2 \pm 5.4 pg/islet per h, n=7), while it was enhanced 6-fold in W islets (33.8 \pm 5.8 pg/islet per h, n=6; $p < 0.001$ vs 10 mmol/l arginine).

Conclusions: PTPase activities in the A-cell may play important but complex roles in the regulation of glucagon release, since inhibition of PTPase activity by POV - depending on the concentration of the compound - either stimulates or inhibits the release. The potent inhibition of arginine-stimulated glucagon secretion by 100 μ mol/l POV in GK rat islets suggests abnormal PTPase activity behind the augmented A-cell secretion in diabetes.

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PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE MIMICKS THE ATP-SENSITIVE K-CHANNEL-INDEPENDENT ACTION OF GLUCOSE AT LOW GLUCOSE CONCENTRATIONS

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Background and Aims: Pituitary-adenylate cyclase activating peptide (PACAP) is released from intrapancreatic nerve endings. PACAP increases cytosolic cAMP and results in receptor knock-out mice indicate its participation in phasic insulin secretion in vivo. Here we have explored how PACAP interacts with the ATP-sensitive K channel (KATP channel)-independent stimulation by glucose on insulin secretion.

Materials and Methods: The patch-clamp technique was used to monitor electrical activity and Ca²⁺-currents in clonal INS-1 cells, with exocytosis monitored as increases in cell capacitance.

Results: PACAP (100 nM) had minimal effects on glucose-induced electrical activity. We then explored the glucose-dependence of PACAP-mediated actions on voltage-gated Ca²⁺ currents and exocytosis elicited by single 300 ms voltage-clamp depolarisations. At 3.3, 10 and 20 mM glucose, exocytosis amounted 20 \pm 4, 40 \pm 5 and 81 \pm 17 fF, respectively ($P < 0.05$; n=24[3.3] & 25[10]; and $P < 0.05$; n=25[10] & 12[20]). Since Ca²⁺-influx was unaffected, these results demonstrate the KATP channel-independent action of glucose. PACAP stimulated exocytosis significantly at all glucose concentrations, but the effect was relatively largest at 3.3 mM glucose and was 180% ($p < 0.001$; n=24[control] & 17[PACAP]). PACAP also augmented depolarisation-elicited Ca²⁺-influx. The Ca²⁺ current vs. exocytosis relationship was best described by a linear function, enabling us to use the ratio k of the two to quantify the Ca²⁺-efficacy of exocytosis. This comparison confirmed that: a) glucose increased the Ca²⁺-efficacy of exocytosis in a concentration-dependent manner ($p < 0.01$; n=24[3.3] & 12[20]); and, b) PACAP mimicked this effect only at 3.3 mM glucose ($p < 0.05$; n=24[control] & 17[PACAP]). At higher glucose concentrations PACAP primarily acted by further increasing voltage-dependent Ca²⁺ influx. The effects of PACAP were largely suppressed by Rp-8-Br-cAMPS, indicating the involvement of protein kinase A. A role for PKA in the KATP channel-independent action of glucose was suggested by the findings that: 1) the phosphatase inhibitor okadaic acid (100 nM) itself increased exocytosis at 3.3 mM glucose by 92 \pm 19% ($P < 0.01$; n=5), and 2) that Rp-8-Br-cAMPS reduced the glucose mediated potentiation of Ca²⁺-dependent exocytosis by >60% ($P < 0.05$; for 3.3 to 20 mM, n=5).

Conclusions: These results suggest that PACAP mimicks the KATP channel-independent actions of glucose at low glucose concentrations, whereas at higher glucose concentrations this effect is not operative. Furthermore, PKA plays a pivotal role for both the PACAP- and glucose-mediated actions.

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ACTIVATION OF AN INWARD Na^+ CURRENT BY ACETYLCHOLINE IN MOUSE PANCREATIC β -CELLS.J.F. Rolland, J.C. Henquin and P. Gilon. *Unité d'Endocrinologie et Métabolisme, University of Louvain, Brussels, Belgium.*

Our aim was to identify and characterize the current by which acetylcholine (ACh) depolarizes the plasma membrane of pancreatic β -cells. The observations that this depolarization is abrogated by omission of Na^+ , and that ACh increases cytosolic $[\text{Na}^+]_i$ suggested that the current is carried by Na^+ . **Methods:** Both conventional and perforated whole-cell modes of the patch-clamp technique were used in single mouse β -cells, voltage-clamped at -80 mV. **Results:** In the presence of external Na^+ , ACh induced a sustained inward current in intact β -cells (perforated mode), which was reversible upon washout of ACh and fully prevented by the muscarinic antagonist atropine. The maximal current was 0.77 ± 0.15 pA/pF at 100 $\mu\text{mol/l}$ ACh, and the EC_{50} was observed at ~ 2.4 $\mu\text{mol/l}$ ACh. This current was not mediated by the rise in $[\text{Ca}^{2+}]_i$, nor by intracellular Ca^{2+} pool depletion because it was unaffected by pretreatment of the cells with thapsigargin (perforated mode) or by addition of 10 mmol/l EGTA to the pipette solution (conventional whole-cell mode). Abrogation of the current by omission of Na^+ from the medium suggests that the current activated by ACh carries Na^+ . Surprisingly, ACh failed to induce an outward Na^+ current when the electrochemical gradient for Na^+ was reversed (Na^+ -rich pipette solution and Na^+ -free bath medium). It did not activate any outward K^+ current either (K^+ -rich pipette solution and (Na^+, K^+)-free bath medium). Insensitivity of the ACh-activated Na^+ inward current to tetrodotoxin excludes the participation of voltage-dependent Na^+ channels. When the pipette solution contained GTP- γ -S or GDP- β -S instead of GTP, ACh still activated a reversible inward current, suggesting that the current is not activated through a G protein. **Conclusions:** Muscarinic receptor activation elicits a Na^+ current in pancreatic β -cells. By depolarizing the plasma membrane, this current contributes to open voltage-dependent Ca^{2+} channels, increase $[\text{Ca}^{2+}]_i$, and eventually trigger exocytosis.

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DETERMINATION OF THRESHOLD GROWTH HORMONE CONCENTRATION REQUIRED FOR INDUCING BENEFICIAL EFFECT ON INSULIN SECRETION FROM HUMAN FETAL ISLETS DURING LONG-TERM INCUBATION

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Background and Aims: In our previous studies we have shown that long-term incubation (15 days) with growth hormone (GH) has a beneficial effect of preventing the decline of insulin secretion capacity from isolated human fetal islets during cultivation in vitro, but the ability of low GH concentrations to induce this effect remains to be clarified. Therefore, the aim of this study was to determine the threshold GH concentration required for achieving the beneficial effect of the long-term GH incubation.

Materials and Methods: The islets were isolated by collagenase digestion, and cultured in media with 10% fetal calf serum at 37°C , 5% CO_2 . The effect on insulin secretion was evaluated by using a 15 day incubation of islets with 200, 300, 400, 500, 600 and 1000 $\mu\text{g/l}$ GH (Genotropin, Kabi Pharmacia) as well as without GH. The insulin secretion capacity was evaluated by determining insulin levels in culture media after 1 hr incubation sequentially with 1.67 mmol/l glucose and 16.7 mmol/l glucose + 5 mmol/l theophylline, and expressed as a percentage of the increase in insulin levels after stimulation.

Results: We found that insulin response remained stable with 400 $\mu\text{g/l}$ GH (day1: 767.9 \pm 64.3%, day15: 761.6 \pm 69.7%; $p = \text{NS}$) and the response pattern did not differ significantly when higher doses of 500, 600 or 1000 $\mu\text{g/l}$ GH were applied. In contrast, the insulin response declined with 200 (day1: 237.3 \pm 29.3, day15: 124.2 \pm 19.2%; $p < 0.01$) and 300 $\mu\text{g/l}$ GH (day1: 421.6 \pm 38.2, day15: 213.4 \pm 28.1%; $p < 0.01$) showing the response pattern similar to that of the islets incubated without GH (day1: 223.5 \pm 24.7%, day15: 118.1 \pm 14.3%; $p < 0.05$).

Conclusions: Our results have demonstrated a threshold GH concentration required for its potentiating effect on insulin secretion from isolated human fetal islets which might be of relevance during islet preparations for clinical transplantation

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The prevalent Gly1057Asp polymorphism in the insulin receptor substrate -2 gene is not associated with impaired beta cell function or development

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Background and Aims: Disruption of the insulin receptor substrate (IRS)-2 was shown to cause type 2 diabetes in mice. This could be largely attributed to abnormal beta cell development. In humans, a prevalent polymorphism in IRS-2 (Gly1057Asp) was not found to be associated with type 2 diabetes in linkage and association studies. We tested the hypothesis that an extreme challenge of the beta cell might reveal subtle abnormalities in carriers of this polymorphism undetected by conventional insulin secretion tests.

Materials and Methods: In addition to assessing beta cell function by oral glucose tolerance testing (OGTT) ($n = 318$, normal glucose tolerance) we measured the secretory response to maximal stimulation by hyperglycemia (10 mM), glucagon-like-petide (GLP)-1 and arginine administered in an additive fashion ($n = 77$, non-diabetic). The allelic frequency of the Asp allele was $\sim 37\%$.

Results: Neither the beta cell function indexes from the OGTT nor the secretory response during the hyperglycemic clamp differed measurably between carriers and controls. Moreover, maximal plasma C-peptide concentrations in response to the combined glucose, GLP-1 and arginine stimulus was not different between Gly/Gly (10745 \pm 1186) and X/Asp (10800 \pm 490 pmol/l, $p = 0.99$).

Conclusions: Our findings strongly suggest that the Gly1057Asp polymorphism in IRS-2 is not associated with beta-cell dysfunction. The normal maximal insulin secretory response strongly indicates that this common polymorphism does not result in developmental defects of the endocrine pancreas or reduced beta cell mass.

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MELATONIN RECEPTOR EXPRESSION BY HUMAN AND RODENT β -CELLS: FUNCTIONAL ROLE IN INSULIN SECRETION?

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Background and Aims: Melatonin secreted by the pineal gland entrains circadian rhythms in many physiological processes through interaction with two G-protein-coupled receptors (MTs). We have now investigated whether pancreatic islets and β -cells express MTs and whether melatonin influences β -cell function. **Materials and Methods:** Expression of MT mRNA and protein by human and rat islets and MIN6 β -cells was determined by RT-PCR and Western blotting, as appropriate. Changes in intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) were assessed by microfluorimetry and insulin secretion from perfused human islets and MIN6 cells was measured by radioimmunoassay. **Results:** RT-PCR using primers specific for MT₁ and MT₂ receptors amplified single products of the appropriate sizes from mRNA isolated from rat (360 and 154bp) and human islets (321 and 285bp). Receptor expression was confirmed in β -cells using RNA isolated from MIN6 cells. An anti-MT₁ receptor antibody recognised the 60kDa MT₁ receptor in MT₁ receptor-overexpressing fibroblasts and MIN6 cells, and it also detected a 72kDa immunoreactive protein in human islet extracts. Melatonin evoked a dose-related increase in $[\text{Ca}^{2+}]_i$ in MIN6 cells (31% of cells responsive to 1nM melatonin; 73% responsive to 10nM melatonin), that was reversibly inhibited by blockade of voltage-operated Ca^{2+} channels with 10 μM nifedipine (30/30 cells from 5 separate experiments; $P < 0.001$). Melatonin did not affect basal insulin secretion from MIN6 cells configured as pseudoislets (2mM glucose: 105 \pm 2.7% control; +10nM melatonin: 123 \pm 20% control, mean \pm SEM, $n = 3$, $P > 0.2$), but 10nM melatonin caused a small, sustained increase in insulin release from human islets at 2mM glucose (peak: 250% basal, plateau: 180% basal, $n = 2$). **Conclusions:** These data indicate that human and rodent islets and MIN6 cells express MT₁ and MT₂ receptors and that interaction of melatonin with MT₁ and/or MT₂ receptors can initiate insulin secretion from human islets, but not MIN6 cells.

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CONFOCAL IMAGING OF INSULIN GRANULE MOVEMENTS ALONG THE CYTOSKELETON DURING PHASIC INSULIN SECRETION

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Background and Aims: Cumulating evidence suggests that first phase glucose-induced insulin secretion corresponds to the Ca²⁺-elicited discharge of a subset of insulin granules docked to the B-cell plasma membrane. Much less is known about how glucose stimulates granule recruitment during second phase insulin secretion. Here, we have elucidated the role of pre-exocytotic insulin granule translocations in this process.

Materials and Methods: Insulin granules, microtubules and the actin cell cortex were co-labelled using LysoTrackerRed, EGFP-tubulin and EGFP-actin, respectively, and visualised by confocal imaging. We also used the standard whole-cell configuration of the patch-clamp technique to test the effects of potential cytosolic regulators of granule mobility.

Results: Insulin granule motion was intense already in the absence of glucose. Three different types of granule movements could be distinguished: a) random diffusion, b) slow directed movements in the 20–40 nm/s range, and c) fast directed saltatory jumps with velocities between 200 and 2000 nm/s. Double labelling experiments revealed that saltatory jumps occurred exclusively along the microtubules, and disruption of the microtubule network abolished saltatory activity. All three types of movements could be observed sequentially in an individual granule, and before granules were tethered to the cytoskeleton, they diffused for an average 435±90 nm (n=14). Elevating glucose from 0 to 20 mM did not affect the velocity of the fast saltatory jumps or the diffusion coefficient. The slow directed movements were, however, slightly accelerated from 32±1 to 38±2 nm/s (P<0.01; n=22) and this effect was evident within 1 min after glucose elevation. Interestingly, these movements occurred near the plasma membrane where the actin cortex is located and were not microtubule-dependent. We then investigated the effects of cytosolic factors on granule mobility using the standard whole-cell configuration. Immediately after establishment, granule movements were unperturbed, and changes in the intracellular ATP/ADP ratio had no acute effects on granule mobility. However, a time-dependent run-down of granule motion was manifest after >5 min, suggesting that an intracellular constituent required for granule motion was washed out.

Conclusions: Glucose-mediated recruitment of insulin granules for release primarily reflects physical translocation of insulin granules that localise in the peripheral actin network. This effect appears to depend on Ca²⁺-influx from the exterior, rather than a change in the B-cell metabolic state. Microtubule-dependent saltatory activity was not affected by glucose.

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NSF IS PRESENT IN RAT B-CELLS AND IMPORTANT FOR THE RE-LOADING OF THE READILY RELEASABLE POOL.

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Background and Aims: Insulin is characteristically released in a biphasic pattern where the first rapid phase is thought to be attributable to primed granules within a readily releasable pool (RRP), and the second phase to the ATP-dependent mobilisation of granules from a reserve pool. The aim of this study was to investigate the role of NSF (NEM-sensitive factor) in the exocytotic process prior to insulin secretion using a monoclonal antibody against the protein (Mab 2E5).

Material and Methods: For the investigations of the exocytotic process capacitance measurements were combined with the standard whole-cell configuration of the patch-clamp technique. This technique allows an intracellular application of the antibody.

Results: An ~80 kD band corresponding to the size of NSF was found using Western Blot analysis. Immunostaining on B-cells revealed the presence of NSF in the cytosol and NSF was shown to co-localize with insulin, mainly close to the plasma membrane. A train of ten 500 ms depolarising pulses from -70 to 0 mV was applied on single rat B-cells. This allows influx of calcium through voltage-dependent calcium-channels, which triggers exocytosis. The two first pulses in the train can be used to estimate a size of the readily releasable pool whereas the following pulses represent mobilisation of granules from a reserve pool. Under control conditions the RRP was estimated to 227±22 fF (n=6), which was reduced by 35% to 147±18 fF (n=7) in the presence of the antibody. A more pronounced decrease was observed during the latter pulses where an 80% reduction in the increase in membrane capacitance was observed in the presence of Mab 2E5 (11±5 fF vs 57±17 fF under control conditions).

Conclusions: The data suggest that NSF might be involved in an early step of granule recruitment for calcium-dependent exocytosis in rat pancreatic B-cells.

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In vivo imaging of ryanodine receptor-mediated calcium release from dense core secretory vesicles in insulin secreting cells

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Background and Aims: Insulin secretory vesicles represent a large potential store of intracellular Ca²⁺ ions, whose mobilisation could increase cytosolic free Ca²⁺ concentration ([Ca²⁺]_i) and hence contribute to the stimulation of insulin release.

Materials and Methods: In order to measure the free Ca²⁺ concentration in the secretory vesicle matrix ([Ca²⁺]_{SV}), and thus Ca²⁺ flux across the vesicle membrane, we have generated a chimaeric cDNA encoding recombinant aequorin (Aq) and vesicle-associated membrane protein-2 (VAMP2).

Results: In pancreatic islet-derived MIN6 β-cells, [Ca²⁺]_{SV} was 51 ± 7.5 mM (mean ± S.E.M., n = 3), ~5-fold lower than in the endoplasmic reticulum (ER; [Ca²⁺]_{ER}, 249 ± 12.9 mM, n = 3). Secretory vesicle Ca²⁺ uptake was insensitive to inhibitors of the sarco-endoplasmic reticulum Ca²⁺-ATPase (SERCA) pump activity, but sensitive to the P-type Ca²⁺ pump inhibitor, orthovanadate. In contrast to the ER, the vesicular Ca²⁺ store was unaffected by inositol (1,4,5) tris-phosphate in permeabilised cells. However, either caffeine or the ryanodine receptor agonist, 4-chloroethylphenol, induced Ca²⁺ release from the secretory vesicles and ER in intact cells. Cyclic ADP-ribose also decreased both [Ca²⁺]_{SV} and [Ca²⁺]_{ER} in permeabilised cells, an effect potentiated by palmitoyl CoA.

Conclusions: Secretory vesicles sequester Ca²⁺ via an ATP-dependent Ca²⁺ pump, distinct from SERCAs, but with properties similar to that of the yeast Golgi Ca²⁺-ATPase, PMR1 (human ATP2C1). The presence on vesicles of active ryanodine receptors suggests that localised increases in free [Ca²⁺]_i (e.g. close to sites of exocytosis) may activate Ca²⁺ release from vesicles and could contribute to the activation of insulin release by glucose and other agents.

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SNAP-25 PHOSPHORYLATION IN INSULIN SECRETING CELLS

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Background and Aims: The tSNARE SNAP-25 (Synaptosomal Associated Protein of 25 kDa) is necessary for regulated insulin secretion. The present aim was to investigate whether SNAP-25 becomes phosphorylated in insulin secreting cells and if so to further characterise this phosphorylation event.

Materials and Methods: To test for SNAP-25 phosphorylation by PKC, cells were treated with the phorbol ester PMA and analysed by immunofluorescence and Western blotting using an antibody recognising specifically SNAP-25 phosphorylated at residue Ser₁₈₇ (consensus site for PKC phosphorylation). The reversibility of SNAP-25 phosphorylation was studied using a water-soluble PMA homologue, FDA. Phosphorylation of SNAP-25a and b was analysed after expression of the myc-tagged isoforms. GFP-SNAP-25 cysteine mutants, which differ in terms of their palmitoylation and membrane association, were used to study whether SNAP-25 phosphorylation takes place in the cytosol or only when membrane associated.

Results: SNAP-25 is phosphorylated at residue Ser₁₈₇ after treatment of insulin secreting (INS-1) cells with PMA (1 μM). Phosphorylation was detectable after 5 min and increased with time to a maximum after 1 h. SNAP-25a and SNAP-25b isoforms are phosphorylated to the same extent in INS cells. SNAP-25 phosphorylation is rapidly reversible, with little detectable phospho-SNAP-25 30 min after PMA removal and none after 1 h. The soluble (100% cytosolic) GFP-SNAP-25 Cys_{45,58,90,92} mutant (lacking all 4 cysteines necessary for membrane association) was not phosphorylated in contrast to the GFP-SNAP-25 wild-type fusion protein. The single GFP-SNAP-25 Cys₅₈ mutant which is distributed equally between cytosol and membrane, revealed that only the membrane-associated fraction was phosphorylated.

Conclusions: SNAP-25 is phosphorylated at Ser₁₈₇ in insulin secreting cells after treatment with PMA and this phosphorylation is a rapidly reversible event. The results further suggest that SNAP-25 needs to be membrane-anchored in order to be phosphorylated. Studies are presently under way to determine what role phosphorylation of SNAP-25 may play in its biological function and in particular in insulin secretion.

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Phosphodiesterase 3B is activated by glucose and insulin in rat islets and involved in Ca²⁺-stimulated exocytosis

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Background and Aims: cAMP is a potentiator of Ca²⁺-induced insulin granule exocytosis as well as glucose-stimulated insulin release. Selective inhibitors of phosphodiesterase (PDE) 3, an enzyme which catalyzes hydrolysis of cAMP, have been used to increase insulin release in mouse models and human and rat islets. It is conceivable that increased PDE3 activity, on the other hand, may confer inhibition or attenuation of exocytotic and secretory events. Little is yet known regarding which agents are capable of activating B-cell PDE3B. The aim of this study was to investigate activation mechanisms for PDE3B in rat islets and the effect of the PDE3 inhibitor OPC3911 on single B-cell exocytosis.

Materials and Methods: Rat islets were isolated from collagenase-digested pancreas tissue and incubated over night in Krebs Ringer bicarbonate buffer. Following stimulation in the presence or absence of inhibitors, islets were homogenized and PDE3 activity was assayed using 3H-cAMP as substrate. Single B-cell exocytosis was monitored as increases in cell capacitance.

Results: In rat islet incubations, insulin secretion-stimulating concentrations (11-20 mM) of D-glucose increased PDE3B activity by two-fold, whereas incubation with non-metabolizable L-glucose had no effect. The activation was inhibited in the presence of 100 nM wortmannin and 0,1 mM diazoxide, respectively, indicating involvement of phosphatidylinositol 3-kinase and dependence on functional ATP-sensitive potassium channels. Interestingly, a dose-dependent activation of PDE3B was also observed by stimulation of islets with increasing concentrations of insulin (1-100 nM). We then addressed the role of PDE3B in B-cell exocytosis. In intact cells (perforated-patch configuration), OPC3911 increased exocytosis elicited by voltage-clamp depolarisations by 41±11 fF (P<0.05; n=5). Surprisingly, OPC3911 retained a ~30% stimulatory effect when exocytosis was elicited by intracellular dialysis of a Ca²⁺- and cAMP-containing patch electrode solution in the standard whole-cell configuration (P<0.05; n=15 and 17, in the absence and presence of OPC, respectively).

Conclusion: Mechanisms for activation of PDE3B in B-cells are poorly defined; our results indicate both nutrient and hormonal control of PDE3B activity. Local control of cytosolic cAMP concentration by PDE3B, rather than control of global cAMP, may play an important role for modulation of exocytosis in B-cells.

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SELECTIVE INTERACTION WITH EXOCYTOSIS UNDERLIES GLUCOSE-DEPENDENT INSULIN SECRETION BY THE IMIDAZOLINE COMPOUND NNC77-0074

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Aim: To explore the effects of the novel imidazoline compound NNC77-0074 on insulin secretion *in vitro* and *in vivo*. **Methods:** All *in vitro* experiments were performed on intact mouse islets or isolated β -cells. Patch-clamp techniques were used to record electrical activity, ion channel activity and exocytosis. Cytoplasmic Ca²⁺ levels were determined by microfluorimetry. Insulin was assayed by ELISA. Blood glucose and plasma insulin was measured in fasted and postprandial Sprague Dawley rats. **Results:** Intravenous (i.v.) administration of NNC77-0074 (20 mg/kg) significantly increased plasma insulin 2 min later in postprandial rats (from 49.0 pmol/l to 584.5) and lowered blood glucose levels (from 4.8 mmol/l to 3.7 after 30 min, P<0.004). In contrast, in overnight fasted rats NNC77-0074 had no significant effect on plasma insulin (from 6.4 pmol/l to 140.0) or blood glucose levels (from 3.4 mmol/l to 3.0 after 30 min). *In vitro*, NNC77-0074 produced a concentration- and glucose-dependent stimulation of insulin release from intact mouse islets. No stimulation was observed at glucose concentrations \leq 5 mM, whereas a pronounced stimulation (\geq 100%) occurred at higher glucose levels. The insulinotropic action of NNC77-0074 reflected stimulation of Ca²⁺-dependent exocytosis ($>$ 100%; EC₅₀=0.1 μ M), measured as increases in cell capacitance. The ability of NNC77-0074 to stimulate insulin release was not associated with inhibition of ATP-sensitive K⁺ (K_{ATP})-channels, changes in membrane potential, altered cytoplasmic Ca²⁺ levels or increased activity of voltage-dependent Ca²⁺-channels. The stimulatory action of NNC77-0074 was blocked by the PKC inhibitors Calphostin C and Bisindolylmaleimide I but not by the protein kinase A inhibitor Rp-cAMPS. **Conclusion:** These data demonstrate that NNC77-0074 potentiates insulin secretion in a glucose-dependent manner both *in vitro* and *in vivo* by K_{ATP}-channel independent acceleration of Ca²⁺-dependent exocytosis.

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Differential glucose responsiveness in insulin-producing cell lines derived from the INS-1 line is associated with altered Ca²⁺ dynamics and Ca²⁺-evoked exocytosis
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Background and Aims: Insulin secretion is impaired in Type 2 diabetes. To gain insights into the mechanisms of this impairment, we have compared biochemical variables in highly glucose-responsive (line 832/13) and glucose unresponsive (line 832/2) cell lines, which have been derived from INS-1 insulinoma cells by a transfection/selection strategy.

Materials and Methods: Insulin secretion in static incubations was measured by RIA. The cytoplasmic Ca²⁺ concentration [Ca²⁺]_{cyt} was determined by dual wavelength excitation spectrophotofluorimetry, using single cells pre-loaded with FURA-2-AM (45 min). By use of the standard whole-cell configuration of the patch clamp technique, the exocytotic response evoked by intracellular dialysis with a Ca²⁺/EGTA buffer (free cytosolic Ca²⁺ ~2 mM), cAMP (0.1 mM) and ATP (3 mM) was monitored as increases in cell capacitance.

Results: Insulin secretion from 832/13 cells was stimulated 10-fold as glucose was raised from 1 to 15 mM (3.1 ± 0.17 versus 37.7 ± 16 ng/mg/h at 1 and 15 mM glucose, respectively; half-maximal response at 10 mM). In contrast, 832/2 cells maximally increased insulin secretion from 4.8±0.7 to only 7.3±1.3 ng/mg/h under identical conditions. At 15 mM glucose, steady-state [Ca²⁺]_{cyt} was 62±4 nM in 832/13 cells (n=11) versus 40±3 nM in 832/2 cells (n=13; P<0.001); [Ca²⁺]_{cyt} oscillated with 1.4±0.3 pulses/10 min in 832/13 cells (n=11) versus 0.3±0.1/10 min in 832/2 cells (n=13; P=0.013). The oscillatory amplitude was similar in the 832/13 and 832/2 line (93±41 versus 100±22 nM, respectively). Upon membrane depolarization (20 mM K⁺), [Ca²⁺]_{cyt} increased similarly to 228±32 nM (832/13) and 173±15 nM (832/2). Moreover, the Ca²⁺-induced exocytotic response was 2-fold greater in 832/13 than in 832/2 cells (35±7 and 17±5 fF/s, respectively; P<0.01, n=10 and 12).

Conclusions: 832/13 cells exhibit higher steady state [Ca²⁺]_{cyt} and more frequent Ca²⁺ oscillations than 832/2 cells, as well as an exocytotic process that is sensitized to Ca²⁺. These alterations may explain the differential glucose-responsiveness of the novel beta-cell lines.

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ALTERNATIVE MDR SPLICING IS REQUIRED FOR DIRECT POTENTIATION OF EXOCYTOSIS BY SULPHONYLUREAS

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Background and Aims: Hypoglycaemic sulphonylureas (SU) initiate insulin secretion by inhibiting the ATP-sensitive K-channel. However, SU-binding predominantly occurs on intracellular membranes, including those of the insulin-containing granules. We have previously demonstrated that SU also potentiate Ca²⁺-dependent exocytosis in mouse B-cells. This mechanism involves acceleration of granular chloride uptake and facilitation of granular acidification. The process involves a granular 65 kDa protein, related to the multidrug resistance P-glycoprotein (MDR). Here, we have extended on this work and started to characterise the elusive intracellular SU-receptor.

Materials and Methods: Northern blots and rtPCR were performed on isolates from RINm5F and INS-1 cells. SU-mediated stimulation of exocytosis and granule acidification were estimated by capacitance measurements and LysoSensor microfluorimetry, respectively. SU-binding was investigated using photo-affinity labelling (PAL) with [3H]-glibenclamide.

Results: [3H]-glibenclamide binding to a granular 65 kDa protein was demonstrated in RINm5F cells and normal mouse islets, but not INS-1 cells. In RINm5F cells, tolbutamide (100 μ M) stimulated exocytosis elicited by intracellular dialysis of a Ca²⁺-containing patch electrode solution (free cytosolic [Ca²⁺]_i 170 nM) by 92% (P<0.01; 21±/8 and 12±/4 fF/s; n=8 & 7, with & without tolbutamide, respectively). This effect was paralleled by a SU-mediated ~50% stimulation of granule acidification (P<0.05; n=8, in both groups). By contrast, in INS-1 cells SU failed to stimulate either exocytosis or granule acidification. The SU-mediated stimulation of exocytosis could be prevented by JSB-1 or C219, functional antibodies directed against the MDR. These Ab's recognise a 65 kDa protein, possibly representing a splice variant of MDR, which lead us to explore MDR expression on the RNA level. In a Northern blot using a probe against MDR we detected a 4600 bp band corresponding to full-length MDR in both cell lines. A prominent 2400 bp band was detected in RINm5F cells, which can be predicted to produce a 65 kDa protein. Interestingly, this band was absent in INS-1 cells. We confirmed the expression of full-length MDR in both RINm5F and INS-1, and a truncated transcript in RINm5F by rtPCR.

Conclusions: These results indicate that the SU-mediated potentiation of granule acidification and exocytosis requires alternative MDR splicing. We speculate that the granular SU-binding 65 kDa protein represents a splice product of MDR.

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IMIDAZOLINES STIMULATE Ca^{2+} -INDUCED EXOCYTOSIS FROM SINGLE PANCREATIC β -CELLS BY A PLA_2 -DEPENDENT MECHANISM

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Aim: To explore if the stimulatory action of imidazoline compounds on exocytosis is dependent on phospholipase A_2 (PLA_2) activity. **Methods:** Exocytosis was measured in single mouse pancreatic β -cells as increases in cell capacitance using the patch-clamp technique. Cytoplasmic free Ca^{2+} -levels were determined by microfluorimetry. **Results:** Infusion of the imidazoline compound NNC77-0074 (100 μ M) into isolated β -cells strongly stimulated Ca^{2+} -dependent exocytosis (from 5.08 ± 0.88 to 9.0 ± 0.3 fF/s, $P < 0.001$). This stimulatory action of NNC77-0074 on exocytosis was found not to be associated with inhibition of ATP-sensitive K^+ -channels or changes in cytoplasmic free Ca^{2+} -levels. A similar stimulation of exocytosis by NNC77-0074 (100 μ M) was observed in INS-1E insulinoma cells. Application of HELSS (30 μ M) or ACA (100 μ M), inhibitors of PLA_2 , abolished the stimulatory action of NNC77-0074 on exocytosis (from 9.2 ± 1.3 fF/s to 4.8 ± 1.1 fF/s, $P < 0.05$ and 5.06 ± 0.88 fF/s, $P < 0.05$). Since neither HELSS nor ACA is specific for PLA_2 INS-1E cells were transfected with anti-sense against cytoplasmic PLA_2 type IV (5'-GTGCTGGTAAGGATCTAT-3'), PLA_2 sense (5'-GTGCTCCTAAGTTCTAT-3') or unrelated oligonucleotides to confirm the PLA_2 dependence of NNC77-0074-induced exocytosis. NNC77-0074-stimulated exocytosis was completely blocked to background levels (sense transfected cells without NNC77-0074 stimulation) by anti-sense PLA_2 transfection, 2.1 ± 0.4 fF/s and 2.0 ± 0.4 fF/s respectively. This was not due to a general impairment in exocytosis since the anti-sense treated INS-1E cells responded normally to high Ca^{2+} or tolbutamide-induced exocytosis. Insulin release measured on transfected INS-1E cells correlated with the exocytosis data. **Conclusion:** These data demonstrate that NNC77-0074 stimulates Ca^{2+} -induced exocytosis through a PLA_2 -dependent mechanism, without interfering with the K_{ATP} -channel activity.

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Insulin Release in Vitro

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ISOLATION AND STRUCTURAL CHARACTERISATION OF INSULINOTROPIC PEPTIDES FROM *BOMBINA VARIEGATA*

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Aims: To isolate and characterise insulinotropic peptides from the skin secretions of *Bombina variegata* frogs. **Methods:** Crude venom (5 mg; 5-10 frogs) obtained by mild electrical stimulation from the dorsal skin surface was purified by reversed-phase HPLC on a semi-preparative Vydac C18 column. Peaks were assayed for insulin-releasing activity by acute 20 min incubations with glucose-responsive BRIN-BD11 cells. **Results:** Three prominent fractions from a total of 44 showed 1.4-3.3-fold increases in insulin-release ($p < 0.001$, $n=3$) compared with control (5.6 mmol/l glucose alone). The viability of BRIN-BD11 cells assessed by neutral red assay (16 mmol/l alloxan as positive control) was not affected under these conditions. Electrospray mass spectrometry analysis of the three major peaks revealed molecular masses of 1641.7, 1662.6 and 1619.8 Da respectively. The amino acid sequences of these purified insulin-releasing peptides were successfully completed in a single analysis revealing peptide structures each with 14 amino acids. The database search of the amino acid sequences of purified insulin-releasing peptides revealed 100% homology of 1619.8 Da peak with bombesin. The two other peaks 1641.7 Da, and 1662.6 Da represented entirely novel peptides with 73-93% resemblance to native bombesin. To evaluate the mechanism of action of the insulin-releasing peptides, acute 20 min incubations were performed with each peptide alone or in the presence of either 300 μ M/liazoxide, 50 μ M/l verapamil or 30 mmol/l KCl. The stimulatory effects induced by the 1641.7 Da peptide on insulin release were reduced by 92% by liazoxide ($p < 0.001$, $n=4$), whereas the stimulatory effects of other two peptides were abolished. Verapamil induced a 47% reduction in the secretory response to bombesin (1619.8 Da peptide) but had no effect on responses to 1641.7 and 1662.6 Da peptides. All three peptides maintained their ability to enhance insulin release in cells depolarised with 30 mmol/l KCl ($P < 0.001$, $n=4$). **Conclusion:** The skin secretions of *Bombina variegata* frogs contain novel peptides with insulin-releasing activity, which may be useful for exploitation of antidiabetic agents.

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FATTY ACYL COA ESTERS ARE REQUIRED FOR THE IMIDAZOLINE INSULIN SECRETAGOGUE EFAROXAN TO INITIATE INSULIN SECRETION

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Background and Aims: The imidazoline efaroxan (efx) can potentiate insulin secretion, but it fails to increase release when islets are incubated in the absence of glucose. The secretory effects of efx involve both the blockade of K -ATP channels and the regulation of more distal events in exocytosis, but it is not clear why the response exhibits such a strict glucose-dependence. A recent hypothesis has proposed that long chain fatty acyl-CoAs (LCCoAs) are required for full induction of nutrient-induced insulin release. Thus, in this study, we have investigated whether efx can initiate insulin release in the absence of glucose by manipulation of intracellular free fatty acid levels.

Materials and Methods: Islets of Langerhans were isolated from Wistar rats and used in static incubation secretory studies. Insulin release was determined by RIA.

Results: At zero glucose, islets responded to palmitic acid (PA) with a dose-dependent increase in insulin secretion, but failed to respond to efx (up to 100 μ M) under these conditions. By contrast, when incubated in the presence of a non-stimulatory concentration of PA (10 μ M), rat islets responded to 100 μ M efx with a robust increase in insulin secretion (efx alone: 0.52 ± 0.05 ; efx plus PA: 0.99 ± 0.02 ng/islet/h, $p < 0.001$). This response was attenuated by 150 μ M cerulenin, an inhibitor of protein acylation (0.70 ± 0.03 , $p < 0.001$) and by the fatty acyl-CoA synthetase inhibitor triacsin C. eAMP has been shown to increase intracellular LCCoA levels but, in the absence of glucose, the adenylate cyclase activator, forskolin (5 μ M) failed to enhance insulin release. However, co-administration of forskolin with efx resulted in an increase in insulin secretory rate (from 0.51 ± 0.03 to 1.05 ± 0.07 ng/islet/h, $p < 0.001$), and this was antagonised by cerulenin (0.76 ± 0.04) and by triacsin C (0.58 ± 0.05). The imidazoline insulin secretagogue antagonist KU14R also blocked the synergistic secretory response induced by forskolin and efx (0.76 ± 0.05). Equivalent results were obtained when 100 μ M IBMX was used in place of forskolin.

Conclusions: In the absence of glucose, efx can initiate insulin release under conditions when intracellular levels of LCCoAs are elevated. Blockade of fatty acid activation or protein acylation antagonises this response suggesting that acyl-CoA molecules may represent critical coupling factors that are required for imidazoline-induced insulin secretion.

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GHRELIN INHIBITS INSULIN SECRETION IN MICE.

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Background and Aims: The peptide ghrelin is expressed in stomach oxyntic cells and is the endogenous ligand for the growth hormone secretagogue receptor. Administration of ghrelin increases food intake and lowers energy expenditure, causing adiposity in rodents. Furthermore, ghrelin plasma levels correspond to the nutritional state in rats, being highest during fasting; thus ghrelin might be involved in regulating metabolism. Our aim was to test the effects of ghrelin on glucose-stimulated insulin release in normal and glucose-intolerant mice, the latter exhibiting hyperglycemia and hyperinsulinemia caused by high-fat diet.

Materials and Methods: Healthy C57Bl/6J mice were given ghrelin (0.5 nmol/kg) during an in vivo glucose tolerance test (IVGTT, 1 g D-glucose/kg). Insulin secretion from islets isolated from C57Bl/6J mice was tested in the presence of ghrelin.

C57Bl/6J mice were given a high-fat diet (58 % fat) or a control diet (11% fat) for 9 months, whereafter animals were injected daily with ghrelin (50 nmol/l i.p.) or saline for 7 days. Insulin response to a glucose stimulus was assessed by IVGTT on day 8.

Plasma insulin was measured by RIA, glucose levels by the glucose-oxidase method. Statistics were calculated by ANOVA.

Results: Ghrelin caused a significant inhibition of the acute insulin response to iv glucose (179 ± 61 pmol/l (50 nmol/kg ghrelin), vs. 573 ± 83 pmol/l in controls). An inhibitory effect on insulin secretion was also seen in isolated islets, where insulin secretion was greatly reduced (by 77%) in 22.2 mmol/l glucose (384 ± 104 pmol/l vs. 1650 ± 503 pmol/l in controls; $p < 0.001$), when ghrelin was present at 1 nmol/l in the medium. After 9 months on high-fat diet, mice displayed weight increase, hyperglycemia and hyperinsulinemia compared to control mice (54.3 ± 1.4 vs. 26.6 ± 0.5 g; 10.2 ± 0.5 vs. 3.1 ± 0.3 mM; and 195.1 ± 29.7 vs. 34.5 ± 5.4 pmol/l, respectively, all $p < 0.001$). Seven days of ghrelin administration did not affect these parameters. However, reduced insulin secretion after iv glucose in the group fed with normal diet (20 min insulin: 391 ± 58 vs. 917 ± 264 pmol/l, $p = 0.02$) in association with impaired glucose elimination in ghrelin-treated high-fat fed mice (20 min value 32.3 ± 0.9 vs. 26.0 ± 3.0 mmol/l ($p = 0.048$) was observed after ghrelin treatment.

Conclusions: Ghrelin potentially inhibits insulin release in response to a glucose stimulus in healthy mice. Furthermore, in already glucose-intolerant, high-fat diet fed mice, ghrelin further reduced glucose tolerance. Since ghrelin is associated to fasting, our results suggest that this peptide contributes to the islet adaptation to fasting.

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Inhibition of tetrahydrobiopterin synthesis partially reverses IL-1 induced inhibition of insulin secretion in rat islets of Langerhans

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Background and Aims: Nitric oxide generated following cytokine induction of iNOS is thought to mediate both functional inhibition and cytotoxicity in pancreatic beta cells. We have shown previously that synthesis of the iNOS cofactor tetrahydrobiopterin (BH4) is required for maximal IL-1 β -induced NO synthesis in rat islets of Langerhans. The aim of this study was to assess the effects of the BH4 synthesis inhibitor 2,4-diamino-6-hydroxy-pyrimidine (DAHP), on IL-1-induced inhibition of insulin secretion. **Materials and Methods:** Rat islets of Langerhans were cultured for 48h and then treated +/- IL-1 (20U/ml) +/- DAHP (5mM) +/- sepiapterin (an alternative substrate for BH4 synthesis, 50uM) for 24h. Islets were then pre-incubated in 2mM glucose for 30min prior to a challenge with 20mM glucose for 1h. Insulin secreted was measured by radioimmunoassay. **Results:** Insulin secretion in control islets was significantly increased following challenge with 20mM glucose (2mM 0.61 +/- 0.12ng insulin/islet, 20mM 1.74 +/- 0.11ng insulin/islet, mean +/- SEM, n=5, p<0.0002). Treatment of islets with 20U/ml r.human IL-1 abolished this glucose-induced insulin secretion (2mM 0.34 +/- 0.06ng insulin/islet, 20mM 0.37 +/- 0.05ng insulin/islet, n=5). The BH4 synthesis inhibitor DAHP significantly reversed the inhibitory effect of IL-1 on glucose-stimulated insulin secretion (IL-1 + DAHP: 2mM 0.29 +/- 0.04ng insulin/islet, 20mM 0.72 +/- 0.12ng insulin/islet, n=5, p<0.01). When sepiapterin, which acts as a substrate for BH4 synthesis via a salvage pathway, was included the effect of DAHP on cytokine-inhibited insulin secretion was attenuated (IL-1 + DAHP + sepiapterin: 2mM 0.38 +/- 0.09ng insulin/islet, 20mM 0.49 +/- 0.17ng insulin/islet, n=4). **Conclusions:** The effects of the BH4 synthesis inhibitor DAHP on insulin secretion correlate with our previous findings showing that this compound significantly inhibits IL-1-induced NO generation in rat islets. These findings support the hypothesis that IL-1-induced, NO-mediated inhibition of insulin secretion is dependent upon synthesis of tetrahydrobiopterin. Synthesis of the NOS cofactor tetrahydrobiopterin therefore represents a possible target for intervention in cytokine-induced NO-mediated dysfunctional effects on beta cells.

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Effects of chronic exposure of isolated human islets to glimepiride, glibenclamide or chlorpropamide

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Background and Aims: The direct effects of prolonged exposure to sulphonylureas on the function and survival of human islets (HI) are unknown. This study assessed insulin content, glucose-stimulated insulin release and the occurrence of apoptosis in HI cultured in the presence of glimepiride (Gp), glibenclamide (Gb), or chlorpropamide (Chl).

Materials and Methods: HI were prepared by collagenase digestion and density gradient purification from 10 multiorgan donors, and were then exposed for 24h to Gp (1.0 or 10 μ M), Gb (1.0 or 10 μ M) or Chl (200 or 600 μ M). Insulin content (IC, by acid extraction), glucose (G)-stimulated insulin release (expressed as % of IC) and the occurrence of apoptosis (by an ELISA technique) were evaluated.

Results: IC in control HI was 142 \pm 41pU/islet and was not significantly affected by 1.0 μ M Gp (81 \pm 18% of control HI). IC was significantly lower after exposure to 10 μ M Gp (64 \pm 10% of control HI), 1.0 and 10 μ M Gb (58 \pm 15 and 69 \pm 43%), 200 and 600 μ M Chl (49 \pm 17 and 44 \pm 22%). Insulin release from control HI in response to 45 min challenge with 3.3 and 16.7 mM glucose was 2.6 \pm 0.3 and 5.3 \pm 1.9% (p<0.01). This glucose responsiveness was maintained in HI pre-exposed to 1.0 μ M Gp (2.8 \pm 0.3 and 4.1 \pm 0.7% respectively at 3.3 and 16.7 mM glucose, p<0.01), 10 μ M Gp (3.4 \pm 1.0 and 4.6 \pm 1.7%, p<0.01), and 200 μ M Chl (2.6 \pm 0.5 and 3.5 \pm 0.8%, p<0.05); on the contrary, HI pre-cultured with 1.0 μ M Gb, 10 μ M Gb, and 600 μ M Chl showed no increase of insulin release at 16.7 vs 3.3 mM glucose. The amount of apoptotic cells (expressed as arbitrary OD units) did not differ significantly between the experimental groups, and ranged 1.3 \pm 0.4 to 2.3 \pm 0.6.

Conclusions: Minimal disturbances of human islets function occurred after prolonged culture with glimepiride, as compared to the alterations observed following chlorpropamide and, more markedly, glibenclamide exposure. These *in vitro* results raise the possibility that glimepiride may have less adverse effects on pancreatic beta-cells during treatment of type 2 diabetic patients.

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Tetracycline-Regulated Secretion of Mature Human Insulin in Transfected Primary Rat Myoblasts.

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Background and Aims: Long term constitutive secretion of insulin by host skeletal muscle following transduction with an insulin gene construct offers a potential approach to gene therapy for diabetes mellitus, circumventing the need for immunosuppression. Transfection may be performed *ex vivo* in primary myoblasts derived from muscle biopsy with subsequent reimplantation by intramuscular injection or *in situ* by direct injection of naked plasmid DNA. Therapeutic applicability will however be critically dependent on a mechanism for regulating insulin secretion. The aim was to evaluate the potential for tetracycline-responsive transcriptional control of human insulin secretion in primary muscle cell cultures.

Materials and Methods: The human cDNAs for wild-type proinsulin (hpi-1) and a mutated construct (hpi-4), in which the dibasic PC2 and PC3 cleavage sites were altered to form tetrabasic consensus sites for furin, were sub-cloned into a tetracycline-responsive plasmid (pTRE). Transient transfections of the constructs with another plasmid, pTet-Off (coding for a transactivating protein) were performed in primary myoblast cultures derived from adult Sprague Dawley rat soleus muscle. Secreted pro/insulin levels were measured using a non-specific antibody, measuring total insulin-like immunoreactivity and a specific antibody, measuring fully processed insulin.

Results: In the absence of tetracycline, peak pro/insulin secretion was attained at 24-48 hours post-transfection for hpi-1 (1.1 ng/ml/24 hours) and at 48-72 hours post-transfection for hpi-4 (0.338 ng/ml/24 hours). Conversion of proinsulin to insulin was low with hpi-1 transfected cells (<20%) compared to hpi-4 (>80%). Tetracycline was added to fresh medium at the start of each 24 hour incubation period. In the presence of increasing concentrations of tetracycline (0.001, 0.01 and 0.1 μ g/ml), pro/insulin secretion was reduced to 66, 48 and 42% for hpi-1 and 64, 47 and 39% for hpi-4, respectively compared to untreated cells (100%) during the 24-48 hour incubation. Following an additional 24 hour incubation (48-72 hour) with equivalent tetracycline concentrations, pro/insulin secretion was reduced further to 67, 22 and 9% for hpi-1 and 75, 32 and 11% for hpi-4 transfections compared to untreated cells (100%).

Conclusions: Regulated secretion of fully processed human insulin has been attained in primary muscle cell cultures following *in vitro* transient transfection with a tetracycline-responsive insulin construct.

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Electrical coupling between B-cells in intact islets

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Background and Aims: B-cells within intact islets are electrically coupled. We applied the patch-clamp technique to intact islets in order to study electrical coupling and exocytosis in functionally identified B-cells.

Materials and Methods: All experiments were performed in the perforated patch configuration.

Results: The total electrical coupling of B-cells was estimated to ~1.2 nS by measuring the resting conductance after blockage of KATP-channels and by recording currents leaking in from bursting adjacent cells in the presence of 10 mM glucose. In the presence of TEA (to block outward K⁺-currents), voltage gated Ca²⁺-channels were activated within a few ms at voltages >40 mV. At 15.7 +/- 3.7 mV (n=7) a secondary inward current was activated within ~40-100ms. This current is due to an action potential triggered in a neighbouring cell and was only seen in functionally identified B-cells, not in A- or D-cells. In the presence of 20 mM TEA and 5 mM glucose the resting potential was -68.4 +/- 2 mV. When raising the glucose concentration to 10 mM, B-cells generated action potentials starting at -48 +/- 1.5 mV (n=8). The voltage at which the secondary inward current appears thus indicates when the voltage pulse applied to the patch-clamped cell caused depolarisation from -68.4 mV to -48 mV in the strongest coupled neighbouring cell. From these data the electrical coupling between two cells was calculated to 0.15 +/- 0.01 nS (n=7). Dividing the total coupling conductance with this estimate gives that B-cells are electrically coupled to 7-8 cells. Application of voltage pulses to -55 mV from a holding potential at -70 mV elicited a slow capacitive current with a time constant of 13.8 +/- 1.7 ms (n=5) due to charging of coupled cells. This slow component was neither detectable in A- or D-cells within intact islets nor in isolated B-cells. This time constant was 7 times larger than that for charging a single cell and does thus not interfere with capacitance measurements. Exocytosis elicited by a voltage-pulse to 0 mV lasting 250 ms was twice as large (n=13) in intact islets as in single B-cells. Voltage pulses to the Ca²⁺-equilibrium potential at +60 mV does not induce Ca²⁺-influx into the voltage clamped cell but triggered action potentials and hence exocytosis in neighbouring cells. Exocytosis in the neighbouring cell was not reflected in an increase of capacitance of the patch-clamped cell.

Conclusions: 1) Only B-cells are electrically coupled. 2) B-cells are coupled to 7-8 cells. 3) Electrical coupling does not interfere with capacitance measurements. 4) B-cells within intact islets have a stronger secretory response than dispersed B-cells.

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IN HUMAN PANCREATIC TISSUE SULFATIDE IS PRESENT EXCLUSIVELY IN BETA CELLS AND IS SEEN IN THE INSULIN SECRETORY GRANULES

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Background and Aims: Anti-sulfatide antibodies have been detected in type 1 diabetes patients and sulfatide has been found in rodent islets. In vitro studies have demonstrated that sulfatide is capable of stabilizing insulin crystals and to promote its monomerization. The aim of the present study was to investigate the localization of sulfatide in human pancreatic tissue and to examine the influence of sulfatide on insulin processing. **Materials and Methods:** Islets including some surrounding exocrine tissue originating from a healthy 41 year old male organ donor were labeled with a sulfatide-specific mAb Sulph I and examined ultra structurally. The tissue was also analyzed for glycolipid content using thin-layer chromatography. Furthermore, the effect of sulfatide on re-folding of denatured proinsulin was investigated in vitro by alkylation of free cystine residues in reduced proinsulin followed by native acryl amide gel analysis. **Results:** Electron micrographs clearly demonstrated the presence of sulfatide in the beta-cell secretory granules, whereas neither alpha cells nor exocrine tissue were labeled. The amount of sulfatide was determined to be 3 pmol sulfatide/ μ g protein, which is comparable to amounts found in rodents. In presence of sulfatide, reduced proinsulin was found to fold momentarily into its native conformation indicated by the formation of proinsulin dimers and hexamers. Without sulfatide proinsulin oligomerization did not occur but remained denatured in its monomeric form. Finally, molecular modeling of the interaction between insulin and sulfatide demonstrates that sulfatide fits surprisingly well to insulin and is compatible with sulfatide folding over the insulin molecule. The dimer-forming surface of insulin is suggested to interact with the fatty acid in sulfatide, and insulin residue A8Thr interacts with the sphingosine base. Hence, this model is in good concordance with our previous data on monomerization. **Conclusions:** In this first study of sulfatide in human pancreatic tissue, sulfatide is found exclusively in beta cells and is seen in high concentrations in insulin secretory granules. In vitro studies with human (pro)insulin show that sulfatide facilitates the folding of proinsulin, preserves insulin crystals, and promotes insulin monomerization.

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Insulin Release in Vivo

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Modelling of Elimination Kinetics of Endogenous Insulin

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Background and Aims: Elimination kinetics of endogenous insulin covering the extreme concentration range have not been determined in humans. The aim of this study was to determine the model parameters which would best predict plasma insulin concentrations using insulin secretion rates (ISR, from plasma C-peptide levels).

Materials and Methods: We performed a modified hyperglycemic Clamp (10 mM Glucose, GLP-1 infusion at 120 min, arginine bolus at 180 min) in 8 normal glucose tolerant (NGT) and 8 impaired glucose tolerant (IGT) subjects. The insulin concentration of a distinct time point was calculated as a function of the following parameters: 1. insulin concentration of the preceding time point, 2. ISR minus hepatic extraction ('first pass effect'), 3. systemic insulin clearance.

Results: In this Clamp insulin concentrations ranged from 72 to 1165 pM (glucose), 376 to 8295 pM (GLP-1) to a peak of 1485 to 15708 pM (arginine). A non-linear, two-compartmental model of insulin distribution was assumed with the following model parameters. 1. Systemic insulin clearance is a sigmoid function of the plasma insulin concentration; 2. Hepatic extraction of secreted insulin ('first pass effect') is a sigmoid function of ISR which serves as semi-quantitative index of portal venous insulin concentrations; 3. Postulation of low affinity binding-sites which cause a time- and concentration-dependent enlargement of the apparent distribution volume at high plasma insulin levels. The difference of predicted vs. measured insulin levels was 20 \pm 10% (glucose), 4 \pm 13% (GLP-1) and 3 \pm 13% (arginine) in NGT and 22 \pm 11, 4 \pm 13 and 3 \pm 12 in IGT respectively.

Conclusions: Our model for insulin elimination is the first one that permits prediction of insulin concentrations from ISR (i. e. C-peptide) over the full physiologic and supraphysiologic range of plasma insulin levels and ISR.

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REPRODUCIBILITY OF PANCREATIC β -CELL RESPONSIVENESS DURING MTT AND OGTT IN HEALTHY SUBJECTS

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Background and Aims: Insulin secretion model (ISM) quantifies fasting (M_0) and postprandial (M_1) prehepatic pancreatic β -cell responsiveness during a meal tolerance test (MTT). We aimed to validate ISM with the oral glucose tolerance test (OGTT) and to compare reproducibility during MTT and OGTT. **Materials and Methods:** Healthy male subjects (N=9, age 27.6 \pm 2.3 year, BMI 24.2 \pm 0.5 kg/m²; mean \pm SE) were studied after a 12 hours overnight fast in random order on four separate occasions one week apart having either a 75g OGTT (2x) or a 500kcal MTT (2x). Fifteen samples were withdrawn every 5-30 minutes over four hours to measure plasma glucose, insulin and C-peptide. **Results:** Validity of ISM with OGTT was assessed and supported by the random distribution of normalised residuals, non-significant results of Runs test, and excellent precision of parameter estimates (CV for M_1 and M_0 < 4%). M_1 and M_0 were not reproducible. Incremental area under curve 0-90 minutes of insulin (ΔAUC_I) but not glucose (ΔAUC_G) or C-peptide (ΔAUC_C) were reproducible. **Conclusions:** In conclusion, ISM was validated during OGTT in healthy subjects. Fasting and postprandial responsiveness were not reproducible due to the lack of reproducibility of glucose and C-peptide profiles. MTT resulted in 1.5 fold higher postprandial pancreatic β -cell responsiveness than OGTT.

	Mean \pm SE		Within subjects variation as % of total variation		Within subjects CV	
	MTT	OGTT	MTT	OGTT	MTT	OGTT
M_1 (10^{-3} \times l/min)	49.4 \pm 5.3*	32.5 \pm 2.8	39%	66%	36%	42%
M_0 (10^{-3} \times l/min)	3.5 \pm 0.5	3.7 \pm 0.3	35%	45%	34%	33%
ΔAUC_G (mmol/l/90min)	88 \pm 11	189 \pm 18	31%	35%	36%	30%
ΔAUC_C (pmol/l/90min)	58.4 \pm 5.4	71.4 \pm 3.9	43%	72%	34%	37%
ΔAUC_I (mU/l/90min)	2.6 \pm 0.4	2.8 \pm 0.4	16%	19%	26%	29%

* P < 0.01, MTT vs OGTT

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Relationships between age, proinsulin conversion and beta cell function in non-diabetic humans

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Background and Aims: Aging is a key determinant of type 2 diabetes. The aim of the present study was to examine the relationships between beta cell function, proinsulin conversion to insulin and age.

Materials and Methods: We studied insulin and proinsulin secretion in non-diabetic subjects during an oral glucose tolerance test (OGTT) using published indices of beta cell function (N=379, age 16 to 68 years) and a modified hyperglycemic clamp (10 mM, additional GLP-1 infusion, final arginine bolus; N=50, age 19 to 68 years). Proinsulin conversion to insulin was assessed using proinsulin/insulin (PI/I) ratios immediately after an acute stimulus (OGT, 30 min; hyperglycemic clamp, 2.5 to 5 min after glucose and arginine).

Results: There was a negative correlation between age and beta cell function (adjusted for insulin sensitivity, BMI, fasting glucose) in the OGTT ($r = -0.21$, $p < 0.001$) and 1st phase of the hyperglycemic clamp ($r = -0.30$, $p = 0.03$) but not 2nd phase ($r = -0.08$, $p = 0.6$) and arginine induced insulin secretion ($r = 0.06$, $p = 0.7$). There was a positive correlation between age and the PI/I ratio in the OGTT ($r = 0.24$, $p < 0.001$). Analogously, there was also a positive correlation between age and the PI/I ratio during 1st phase ($r = 0.37$, $p = 0.009$) and arginine stimulation ($r = 0.33$, $p = 0.01$) of the hyperglycemic clamp. 1st phase insulin secretion of the hyperglycemic clamp was inversely correlated with the PI/I ratio ($r = -0.60$, $p < 0.001$). Interestingly, adjusting 1st phase secretion rate for the PI/I ratio abolished the linear relationship with age ($r = -0.06$, $p = 0.7$).

Conclusions: Aging is associated with deteriorating beta cell function and deteriorating proinsulin conversion to insulin. The age effect on insulin secretion appears to be attributable at least in part to an impairment of proinsulin conversion to insulin.

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FFA ELEVATION MODULATES THE HIGH-FREQUENCY INSULIN SECRETORY PATTERN IN A TIME DEPENDENT MANNER.

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Background and Aims: Free fatty acids (FFA) influence insulin secretion and insulin sensitivity. The present study was undertaken to examine the effect of physiologic acute and short-term elevation of FFA (400 to 600 mmol/L) on high-frequency pulsatile insulin secretion.

Materials and Methods: Eight lean, healthy, young volunteers were examined during acute (1 hour) and short-term (24 hours) infusion of intralipid/heparin compared with infusion of saline. Blood was collected every min for two times 60 min (baseline and glucose entrainment (4 mg/kg body weight every 10 min)) to establish insulin time-series, and finally a first-phase insulin secretion test was performed (25 g glucose iv).

Results: Deconvolution analysis was used to determine basal (non-pulsatile) insulin secretion (BS) and insulin secretory burst mass (BM). Regularity was assessed by spectral and autocorrelation analyses. During entrainment BM significantly increased after acute elevation of circulating FFA (BM: 25.7 ± 5 vs. 45.2 ± 9 pmol/L/min; $p = 0.01$), whereas short-term elevation of FFA significantly increased BS (BS: 6.0 ± 3 vs. 9.0 ± 4 pmol/L/min; $p = 0.005$). No difference was observed in normalized spectral power or autocorrelation coefficient, in the basal or entrainment period, during acute or short term elevation. First-phase insulin secretion, as measured by area under the curve (AUC), was exaggerated during both protocols ($p < 0.05$), hence indicating beta-cell compensation for insulin resistance.

Conclusion: Elevation of circulating FFA does not perturb regularity of high-frequency insulin pulsatility but modulates basal secretion and insulin secretory burst mass in a time-dependent manner.

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The Effect of Fat Infusion on Insulin Secretion in Elderly Obese Man at Increased Risk of Developing Type 2 Diabetes

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Background and Aims: Elevated plasma free fatty acids (FFA) are considered to play an important role for the development of impaired insulin action (IA) and perhaps insulin secretion (IS) in Type 2 diabetic patients.

Materials and methods: We studied the effect of a low grade 0, 2 and 24 hours Intralipid infusion (intended 10% increase of fasting pl-FFA) on IS and IA in 15 elderly obese men, 7 glucose intolerant (IGT) first degree relatives of type 2 diabetic patients (57.2 ± 2 years, BMI 32.0 ± 1.2 kg/m²) and 8 matched healthy controls (53 ± 2 years, BMI 32.7 ± 1.4 kg/m²). Study day A and B were performed. A; an intravenous glucose tolerance test (IVGTT) (0.3 g glucose/kg) to determine the first phase insulin response and a 120 min. euglycaemic hyperinsulinaemic clamp (40mU/m²/min) to determine IA B; a graded glucose infusion to determine the dose-response (dose-response test (DORE)) dependency of pl-glucose and insulin secretion rate (ISR) ending with a 75 min hyperglycaemic (30 mM) clamp combined with arginine injection (arginine test (ARGI)). In order to evaluate IS in relation to IA a disposition index (Di) was calculated ($Di = IA \cdot IS$).

Results: In the total study population and both subgroups IA was reduced app. 30 % from 0 to 2 and 24 hours fat infusion (FI). In the total study population and the subgroup of controls IS wasn't affected by either 2 or 24 hours FI in any of the tests. Within the group of IGTs, however, IS decreased significantly during the IVGTT from 2 to 24 hours of FI ($p < 0.01$). When IA was taken into consideration there was a decrease in Di in the total study population in all tests from 0 to 2 and 24 hours FI, respectively; DiIVGTT (7.85 ± 1.48 vs. 5.14 ± 0.81 vs. 4.98 ± 0.97 ; $p < 0.02$), DiDORE (4.34 ± 0.54 vs. 3.14 ± 0.27 vs. 3.37 ± 0.44 ; $p < 0.02$), DiARGI (1443 ± 331 vs. 770 ± 253 vs. 834 ± 244 ; NS). The two subgroups displayed the same pattern for IVGTT and DORE but the changes were only significant within the group of IGTs; DiIVGTT (0vs24 $p < 0.01$) and DiDORE (0vs 2 $p < 0.03$). DiARGI was decreased near-significantly within the control group ($p < 0.08$).

Conclusions: Fat induced insulin resistance is not balanced by a sufficient compensatory increase in insulin secretion in elderly obese glucose intolerant male relatives of diabetic patients and matched controls.

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INSULIN SECRETION BEHAVIOUR IN TYPE 2 DIABETES AND ABDOMINAL OBESITY

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Background and Aims: We studied the behaviour of the insulin secretion during oral glucose tolerance test (OGTT) in subjects with abdominal obesity, with Type 2 Diabetes obese or non-obese, comparatively with subjects of different age without Diabetes Mellitus or abdominal obesity, but with other diseases. **Materials and Methods:** In the study 2 groups: the first one include 40 normoponderal subjects (20 subjects, without family or individual history of Diabetes Mellitus and 20 subjects with Type 2 Diabetes Mellitus); in the second group we included 40 obese subjects (20 subjects, without family or individual history of Diabetes Mellitus and 20 subjects with Type 2 Diabetes Mellitus). All the subjects were submitted to an oral glucose administration standard test with 75g glucose, at least 12h after the last meal. The glycemia and the insulinemia were measured fasting, at 1, respectively 2 h. **Results:** Fasting insulinemia was 7.90 ± 1.89 µU/ml, increased to 68.1 ± 4.5 µU/ml at 1h and 36.1 ± 2.3 µU/ml at 2h, in normoponderal subjects. The statistical analyse showed a fasting insulinemia significantly higher than (25.68 ± 3.26) and, at 2h (44.5 ± 3.8 ; $p < 0.001$), but less at 1h (39.5 ± 3.2 ; $p < 0.001$), in obese diabetics. In non-obese diabetics, fasting insulinemia was lower (6.51 ± 1.5), comparatively with the non-obese non-diabetics, but not statistically significant. In the non-diabetic obese subjects, fasting insulinemia is significantly higher (25.68 ± 3.26), at 1h (76.3 ± 2.25 ; $p < 0.001$), and at 2h (48.8 ± 2.3 ; $p < 0.001$) comparatively with the normoponderal non-diabetic subjects. **Conclusions:** Abdominal obesity is characterized by an increased level of the fasting insulinemia, at 1 and 2 h. The obese Diabetes Mellitus is characterized by a delayed increase of the maxim insulin concentration, and in non-obese Diabetes Mellitus, by a low insulin secretion at the onset of the disease. The variations of the insulin secretion in subjects without Diabetes Mellitus or abdominal obesity, but with other cardio-vascular, metabolic, renal diseases, suggest variations of the insulin secretion linked to these diseases.

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CHARACTERISTICS OF GLUCOSE STIMULATED INSULIN SECRETION IN CHINESE INDIVIDUALS

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Background and Aims: To investigate glucose stimulated insulin secretion under various glucose tolerant status in Chinese. **Materials and Methods:** 634 individuals (men 286, women 348) aged 58.34 ± 11.32 years were included. Individuals were classified in two ways: 1. Individuals were divided into normal weight (NW) and overweight/obese (OW/OB) group according to the obesity criteria of WHO (1998). Both groups were further divided into 7 subgroups on the basis of fasting glucose levels. 2. Individuals were divided into 6 subgroups according to body mass index (BMI) and glucose tolerant status: NW with normal glucose tolerance (NGT) (NW-NGT), NW with impaired fasting glucose/impaired glucose tolerance (IFG/IGT) (NW-IFG/IGT), NW with diabetes (DM) (NW-DM), OW/OB with NGT (OW/OB-NGT), OW/OB with IFG/IGT (OW/OB-IFG/IGT), OW/OB with DM (OW/OB-DM). Mean insulin concentration during 2 hour OGTT was applied for estimating glucose stimulated insulin secretion. Homeostasis model assessment was used to assess the degree of insulin resistance. **Results:** 1. The relationship between glucose stimulated insulin secretion and fasting glucose concentration was similar to an inverted U-shaped curve. The glucose stimulated insulin secretion reached the peak at fasting glucose level of 5.9 mmol/L in NW subjects. 2. The highest level of the glucose stimulated insulin secretion was observed in OW/OB-NGT group. 3. In OW/OB-IGT group, there was a decrease in glucose stimulated insulin secretion and increase HOMA-IR. 4. OW/OB-DM group was related to a further decrease in glucose stimulated insulin secretion and worsening of the insulin resistance. **Conclusions:** 1. In Chinese individuals the relationship between glucose stimulated insulin secretion and fasting glucose concentration was similar to an inverted U-shaped curve. 2. In the subgroups of NGT, IFG/IGT and DM, there were associated with progressive decrease in glucose stimulated insulin secretion and rise in insulin resistance in Chinese individuals with over weight or obese.

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Acute insulina responses in patients with congenital hyperinsulinemia caused by different K-ATP channel mutations

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Background and Aims: Mutations in the beta cell K-ATP channel genes (SUR1, Kir6.2) are the major cause of congenital hyperinsulinemia (CHI). The clinical course of the disease varies in a remarkable way in patients with different genotypes. In Finland, the SUR1 mutations V187D and E1506K explain 55% of all CHI cases. The aim of this study was to investigate in vivo the acute beta cell responses of CHI patients with different genotypes.

Materials and Methods: Acute (1-5 min) blood glucose, plasma insulin and C-peptide responses to an iv. bolus of calcium, glucose and tolbutamide were investigated in 18 CHI patients (age 1-28 yrs.). K-ATP channel mutations were identified in 12 patients (E1506K, n=6; V187D, n=5; Kir6.2 compound heterozygote, n=1). No K-ATP channel mutations were found in six patients.

Results: Plasma C-peptide and insulin responded significantly better to calcium stimulation in the patients with, as compared without K-ATP channel mutations ($p < 0.05$). The plasma C-peptide responses to calcium were significantly higher in the SUR1 E1506K patient group as compared with the SUR1 V187D patient group ($p = 0.011$). The C-peptide response to tolbutamide was lower in the patients with K-ATP channel mutations ($p = 0.058$). The results of intravenous glucose tolerance test did not differ significantly between the study groups.

Conclusions: Our results show that sudden elevation of the plasma calcium level stimulates insulin secretion in CHI patients with K-ATP channel gene mutations. This result is in accordance with previous studies showing that beta cells with K-ATP channel mutations have a constant activity of voltage-gated calcium channels, possibly explaining why an increase in extracellular calcium could directly stimulate insulin release. An impaired response to tolbutamide is consistent with the existence of a K-ATP channel mutation. These findings support the usefulness of calcium and tolbutamide tests in differentiating the CHI patients with or without K-ATP channel dysfunction.

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ORNITHINE α -KETOGUTARATE POTENTIATES GLUCOSE-INDUCED INSULIN SECRETION *IN VIVO*

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Background and Aims: The anabolic/anticatabolic properties of ornithine α -ketoglutarate (OKG), largely demonstrated in catabolic states, could stem in part from its effects on insulin secretion and/or action. We have previously shown that OKG promotes insulin secretion by perfused and incubated isolated rat pancreatic islets. The aim of this study was to confirm this observation under in vivo conditions and to evaluate its influence on Glucose utilisation. **Materials and Methods:** Because of hypoglycemia induced by OKG in fasted state, non fasted animals were used. Male Wistar rats (250 – 300 g) were injected in a single bolus intravenously either by NaCl 0.9% (C), Glucose (0.8g/kg) (Glc), OKG (12 mg/kg) (OKG12) alone or in the presence of Glc (0.8 g/kg) (Glc/OKG12). Blood samples were collected at t=0, 1, 3, 5, 7, 10, 15, 30, 45, 60 and 75 min. Insulin secretion and glycemia were evaluated. Insulinemia was determined by RIA. Total insulin secretion is appreciated by the area under the curve of insulinemia. Glucose utilisation following glucose load was determined by calculating Kg. Results, presented as mean \pm SEM, were compared using ANOVA and Fisher t-test for comparison of insulin secretion and the non-parametric Mann-Whitney-test for statistical comparison of Kg. **Results:** Insulin secretion in the different groups were as follows:

	AUC (n=10/group)
C	5933 ± 800
Glc	10502 ± 1824^1
OKG12	9240 ± 630
Glc/OKG12	$14204 \pm 710^{1,*}$

¹ $p < 0.05$ vs C, * $p < 0.05$ vs OKG12 and Glc

Glucose utilisation was significantly increased by OKG (Glc: 1.25 ± 0.13 %/min; Glc/OKG12: 1.60 ± 0.05 %/min, $p < 0.05$). At 45 minutes after injection glycemia was indifferent compared to basal values in both groups (t=0 Glc: 9.5 ± 0.4 mmol/l Glc/OKG12 8.9 ± 0.4 mmol/l and t=45 Glc 10.8 ± 0.7 mmol/l Glc/OKG12: 11.1 ± 0.5 , $p < 0.05$ ns). **Conclusions:** Confirming in vitro experiments, OKG stimulates insulin secretion in vivo. Moreover, OKG potentiates glucose induced insulin secretion leading to a significant increase in peripheral glucose utilisation.

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CHRONIC EXPOSURE TO HIGH GLUCOSE OR FREE FATTY ACIDS IMPAIRS INSULIN SECRETION BY INCREASING UNCOUPLING-2 PROTEIN (UCP-2) EXPRESSION AND AFFECTING GLUCOSE METABOLISM.

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Background and Aims: Chronic exposure to high glucose or free fatty acids (FFA) impairs glucose-induced insulin release in vitro. Under these experimental conditions we have recently observed an impairment of glucose oxidation.

Materials and Methods: To further investigate the mechanism of this alteration, we cultured rat pancreatic islets with either high glucose (16.7 mM, 48 h) or high FFA (2mM, 72 h) and then measured glucose oxidation (formation of $^{14}\text{CO}_2$ from U- ^{14}C -glucose), glucose-stimulated Pyruvate Dehydrogenase (PDH) activity (Radiochemical assay: formation of CO_2 from 1- ^{14}C -pyruvate) and Pyruvate Carboxylase expression (by Western Blot analysis). We also measured ATP and ADP levels (bioluminometric assay), and uncoupling-2 protein (UCP-2) expression by Western Blot.

Results: When compared to control islets, islets exposed to high glucose or FFA showed: 1) reduction of glucose (22.0 mM) oxidation (29.8 ± 3.6 or 35.0 ± 2.2 pmol/islet/120 min in islets exposed to either glucose or FFA, respectively, vs 47.5 ± 2.4 in control islets; mean \pm SE, n=4, p<0.01); 2) impaired glucose-stimulated pyruvate dehydrogenase activity (1.28 ± 0.08 μU /islet and 1.49 ± 0.22 in islets exposed to either glucose or FFA, respectively, vs 2.38 ± 0.16 in control islets; mean \pm SE, n=3, p<0.05); 3) unchanged pyruvate carboxylase levels; 4) impaired glucose-induced ATP production (* of increment over baseline: 0.7 ± 0.5 and 0.54 ± 0.3 pmol/islet in islets exposed to either glucose or FFA, respectively, vs 12.1 ± 1.5 in control islets; n=3; p<0.05). As a consequence, glucose-induced increase of the ATP/ADP ratio was reduced (4.7 ± 0.7 and 6.6 ± 0.5 in islets exposed to either high glucose or FFA, respectively, vs 13.8 ± 1.5 in control islets; mean \pm SE, n=3, p<0.01). Moreover, we found that chronic exposure of pancreatic islets to either high glucose or FFA induced overexpression of UCP-2 ($191\% \pm 29\%$ and $197\% \pm 13\%$ in islets exposed to either high glucose or FFA, mean \pm SE, n=4, p<0.05).

Conclusions: These data indicate that in rat pancreatic islets exposed to high glucose or FFA the impairment of glucose oxidation is associated with (and might be due to) a reduction of the ATP/ADP ratio and of glucose stimulated PDH activity, while the expression of UCP-2 protein is increased. Therefore, the rat pancreatic islet response to fuel overload may occur via two different mechanisms: an impairment of ATP production by a reduction of PDH stimulated activity and an increase of energy expenditure by UCP-2 overexpression.

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FATTY ACID INHIBITION OF HUMAN ISLET AND BRIN-BD11 CELL FUNCTION: EVIDENCE OF A ROLE FOR NITRIC OXIDE.

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Background and Aims: Elevated plasma free fatty acid (FFA) levels may play a role in the development of type 2 diabetes. Long-chain FFA alter β -cell function possibly through a nitric oxide (NO) dependent pathway, a mechanism also activated by cytokines. We therefore investigated the effect of FFA and cytokines on insulin release/secretion and NO generation in human islets and BRIN-BD11 cells.

Materials and Methods: Human islets (100 islet equivalents/well), obtained from UK transplant programs, or BRIN-BD11 cells ($\sim 2.5 \times 10^5$ cells/well) were pre-cultured in 24-well plates in RPMI medium for 24 h. The cells were then treated at various glucose concentrations (5.5 , 11 and 22 mmol/l) \pm FFA (2 mmol/l, 2:1, Oleate:Palmitate) or \pm combined cytokines (IFN- γ , IL-1 β and TNF- α , 100 pmol/l each) for 72 and 48 h respectively. Insulin release was measured by RIA, NO generation by Griess assay.

Results: Human islets treated with 2 mmol/l FFA showed a significant ($p < 0.05$) 50-75% increase in insulin release (72-h) at 5.5 and 11 mmol/l glucose, with a significant ($p < 0.05$) 25% decrease observed at 22 mmol/l. However, the insulin released in response to 2 mmol/l FFA appeared maximal (at ~ 6.00 ng/islet/72-h), irrespective of the glucose concentration. Subsequent 1-h insulin secretion was inhibited (~ 10 -fold) by FFA at all glucose concentrations. BRIN-BD11 cells were glucose responsive; FFA significantly decreased insulin release (~ 7 -fold over 48-h) ($p < 0.05$) and secretion (~ 5 -fold 1-h post secretion) ($p < 0.05$). A significant increase in NO generation ($p < 0.05$) ~ 200 -fold was seen in the BRIN-BD11 cells treated with 2 mmol/l FFA, and a 50% increase (N.S.) in human islets.

Conclusions: Incubation with 2 mmol/l FFA caused a stimulation of insulin release (72-h) and inhibition of subsequent acute insulin secretion in human islets. In BRIN-BD11 cells an inhibition of both insulin release and secretion was seen. The results suggest that raised FFA levels not only affect β -cell function in human islets, but also cause a significant decrease in insulin release and secretion in BRIN-BD11 cells. Our results suggest that this may occur via a NO dependent mechanism. This is therefore further evidence that FFA play a role in β -cell dysfunction leading to type 2 diabetes in both human and animal models.

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Lipotoxicity in human pancreatic islets and the effects of metformin.

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Background and Aims: Experiments in rodents have shown that prolonged exposure of pancreatic islets to high concentration of free fatty acids (FFA) causes alterations of insulin release and reduced islet cell survival (lipotoxicity). We evaluated the phenomenon of lipotoxicity in isolated human islets, investigated some of the possible mechanisms and assessed the role of the antidiabetic drug metformin. **Materials and Methods:** Isolated human islets were prepared from multiorgan donors and incubated for 48h in the presence of 2.0 mmol/l FFA (oleate to palmitate, 2 to 1). Insulin secretion was then assessed in response to glucose (3.3 and 16.7 mmol/l), arginine (20 mmol/l) and glyburide (200 μmol /l) during static incubation or by perfusion experiments. Glucose oxidation and utilization, and intraislet triglyceride content were also measured. Finally, the effect of metformin (2.4 μg /ml) was studied.

Results: Glucose (28.3 ± 6.9 vs 56.7 ± 12.6 pmol/islet/45 min, $p < 0.01$), but not arginine (50.4 ± 12.6 vs 47.2 ± 9.4 pmol/islet/45 min) or glyburide (53.5 ± 11.6 vs 44.1 ± 9.4 pmol/islet/45 min) stimulated insulin release was significantly lower from FFA-exposed islets as compared to control islets. Upon perfusion, it was found that impairment of insulin secretion after exposure to FFA was mainly accounted for by a defect of early-phase release (total release: 34.8 ± 5.9 vs 43.8 ± 6.4 pmol/min; 0-10 min release: 19.7 ± 7.2 vs 66.6 ± 16.2 pmol/min; 11-30 min release: 12.6 ± 1.8 vs 17.4 ± 5.4 pmol/min). In control islets, increasing glucose concentration was associated with an increase in glucose utilization and oxidation, which were significantly decreased after incubation with FFA. Islet triglyceride content increased significantly after FFA exposure (25.4 ± 2.5 vs 15.0 ± 2.1 ng/islet; $p < 0.05$). Addition of metformin to high FFA media prevented the impairment of glucose-mediated insulin release, the decline of first-phase insulin secretion, the reduction of glucose utilization and oxidation, without affecting triglyceride accumulation in the human islets. **Conclusions:** These results show that lipotoxicity in human islets is characterized by selective loss of glucose responsiveness and impaired glucose metabolism, with a main defect in early-phase insulin release; metformin prevents these deleterious effects, suggesting a direct protective action on human beta-cells.

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LOCALIZATION OF AMYLOID POLYPEPTIDE IN OTHER ISLET SITES THAN THE SECRETORY VESICLES OF BETA CELLS FROM NON-DIABETIC SUBJECTS.

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Background and Aims: Islet amyloid deposits are present in more than 90% of type 2 diabetic patients but can also occur in the pancreas of non-diabetic subjects. Little is known about the mechanisms which underlie formation of islet amyloid polypeptide (IAPP) fibrils in the islet. The aim of this study was to examine whether sustained stimulation of human beta cells can lead to IAPP fibrils.

Materials and Methods: Isolated islet cell preparations from non-diabetic subjects were cultured for 6 days in Ham's F10 medium at 6 or 20 mM glucose with or without isobutylmethylxanthine as potentiator of the beta cell secretory activity. After culture, cells were fixed and embedded for electron microscopy. Immuno-gold labeling was used to determine the presence of IAPP and N-pro-IAPP at the ultrastructural level. Antibodies against insulin and lysosome-associated membrane proteins (LAMP) were applied to examine localization of the IAPP peptides in secretory vesicles or lysosomes.

Results: In all tested conditions, both IAPP and N-pro-IAPP were found in insulin containing secretory vesicles. In beta cells, IAPP - but not N-pro-IAPP or insulin - was also identified in fibrils and in lipid-storing vesicles, some of which were positive for LAMP. In the islet interstitium, IAPP was often detected on electron-dense structures of cellular debris, which were negative for N-pro-IAPP or insulin. With the present techniques, we were unable to examine any quantitative differences in IAPP distribution among the different culture conditions.

Conclusions: In beta cells from non-diabetic subjects, IAPP is found in other sites than the secretory vesicles, namely lysosomes, cytoplasmic fibrils and extracellular debris. It is still unknown whether environmental conditions influence this distribution, and whether these sites contribute to amyloid formation in vivo.

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PRESENCE OF N-PROISLET AMYLOID POLYPEPTIDE PRECURSORFORM IN ISLET AMYLOID OF TYPE 2 DIABETIC PATIENTS.

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Background and Aims: Amino-acid analysis of islet amyloid in type 2 diabetes has identified islet amyloid polypeptide (IAPP) as its main constituent. Immunochemical analysis has so far not been conclusive for the presence of proIAPP precursor forms. Since we have previously noticed that proIAPP is the main IAPP form released by human beta cells cultured at high glucose levels, we consider the possibility that IAPP precursors might become incorporated in amyloid deposits. We therefore evaluated the occurrence of proIAPP in islet amyloid of type-2-diabetic patients, using different methods of tissue preparation.

Materials and Methods: Human pancreatic tissue, obtained at autopsy from type-2-diabetic patients (n=10) was formalin-fixed, paraffin-embedded and/or unfixed frozen, before sectioning and incubation with polyclonal antibodies to the N- or C-terminal flanking peptide of proIAPP (these antibodies were kindly provided by A. Clark, Oxford, UK). To retrieve antigens, sections were heated in 0.6mol/l citrate buffer. Antibody binding was revealed by fluorescence. Amyloid deposits were visualized by Congo red fluorescence.

Results: In formalin-fixed sections that had not been heated, no C- or N-proIAPP immunoreactivity (IR) was detected in the amyloid deposits. Heating revealed positivity for N-proIAPP but not for C-proIAPP. In frozen sections, no pretreatment was needed to detect N-proIAPP IR on islet amyloid, however, C-proIAPP IR remained negative. N-proIAPP IR was completely blocked by synthetic N-terminal flanking peptide.

Conclusions: Islet amyloid from type-2-diabetic patients contains N-proIAPP but no C-terminally extended precursor form. This observation is compatible with the possibility that increased secretion of N-proIAPP under chronic hyperglycemia results in incorporation of IAPP precursor in islet amyloid of type-2-diabetic patients.

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REGULATION OF THE ENZYME IDURONATE-2-SULFATASE (IDS) IN HUMAN AND MICE PANCREATIC ISLET

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IAPP is the main peptide detected in pancreatic amyloid. Although factors involved in amylin deposition are unknown, the presence of highly sulphated proteoglycans in many forms of amyloid has suggested that they may play a role in amyloidogenesis. The lysosomal enzyme iduronate-2-sulfatase (IDS), involved in proteoglycans degradation, has been identified in human pancreatic islets. A defect in such enzyme, might result in an abnormal proteoglycan metabolism in human islets and this could contribute to amyloid formation. **Aims:** To investigate the expression and regulation of IDS in human and mice pancreatic islets. **Material and Methods:** IDS mRNA expression pattern was analysed by RT-PCR and PCR-in situ hybridization in human pancreatic tissue. Human and mice pancreatic islets were isolated and cultured for 24 hours at 3, 11.1 and 24.4mM Glucose (G). Mice islets were also cultured for 24h in the presence of 24.4mM Mannose (MN), 11.1mM Mannoheptulose (MH), 1mM Glyceraldehyde (GL) or 24.4mM 6-Deoxyglucose (6-DG). IDS and IAPP mRNA levels were quantified by real-time PCR. **Results:** PCR-In situ hybridization and RT-PCR experiments indicate that IDS is expressed in endocrine and exocrine cells. Human pancreatic islets cultured for 24h in the presence of 11.1 and 24.4mM G showed an increase in the IDS mRNA expression of 62% (p<0.001) and 38% (p=0.005) respectively in relation to the control (3mM G). We also observed a 17 fold (p<0.005) and 32 fold (p<0.01) increase in the IAPP mRNA expression at 11.1 and 24.4mM G, using the same control conditions. Mice pancreatic islets cultured at 11.1 and 24.4mM G, showed an increase in IDS mRNA expression of 38% and 120% respectively in relation to the control (3mM G). The addition of GL or 6-DG to the culture medium (at 3mM G) didn't success any effect in the IDS mRNA expression. On the other hand, when we incorporated MN in the same medium, there was an increase in IDS mRNA levels of 75%. The addition of MH to the culture at 24.4mM G elicited a decrease of 98%. **Conclusion:** IDS is located in human pancreatic islets and its mRNA expression is regulated by signals derived from glucose metabolism.

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EFFECTS OF LONG-TERM EXPOSURE TO GLUCOSAMINE ON RAT PANCREATIC ISLET FUNCTION.

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Background and Aims: Since glucosamine competitively inhibits glucokinase activity, its acute addition to isolated islets reduces glucose-induced insulin release. The aim of present study is to investigate the effects of chronic exposure to glucosamine on pancreatic islets.

Materials and Methods: Rat pancreatic islets were cultured in CMRL medium containing 5.5 mM glucose, with or without 10 mM glucosamine, for 24 and 48 hours. At the end of the culture, islets were carefully washed to ensure that glucosamine was completely removed from the medium. We then measured: a) glucose-stimulated insulin release; b) glucokinase activity (by measuring the rate of glucose-6-phosphate formation in a fluorimetric assay); c) glucose utilization and oxidation rates (by measuring the formation of 3H₂O and 14CO₂ from (5-3H)-glucose and (14C)-glucose respectively).

Results: Glucose-induced (22.2 mM) insulin release was unaffected in islets pre-exposed for 24 h to glucosamine (820±109 vs. 883±38 pg/islets/30min in control islets, mean±SE, n=3), but significantly reduced in islets pre-exposed for 48 h to glucosamine (362±104 vs. 787±63 pg/islet/30min, mean±SE, n=3, p<0.05). The islets insulin content was similar in the two experimental groups (30.8±4.8 vs. 39.2±9 ng/islet, mean±SE, n=3). In the islets pre-treated with glucosamine, glucokinase activity was slightly but not significantly reduced as indicated by the enzyme V_{max} (82±10 vs. 111±12 pmol/μg protein/90min, mean±SE, n=4). In contrast, glucose utilization and glucose oxidation were significantly reduced in islets pre-exposed to glucosamine for 48 h when compared to control islets (81±20 vs. 175±20 pMol/islet/120min, p<0.001, and 46±6.4 vs. 65±5.6 pMol/islet/120min, mean±SE, n=4, p<0.05, respectively).

Conclusions: In rat pancreatic islets 10 mM glucosamine reduces glucose metabolism and glucose-induced insulin release only after long-term exposure (48 h). These effects are not dependent on the contemporary presence of glucosamine in the incubation media and on its effect on glucokinase.

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Effects of insulin secretagogues on the viability of pancreatic B-cells

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Background and Aims: The sulfonylurea tolbutamide and several imidazolines have recently been described to affect the viability of pancreatic B-cells and insulin-secreting cell lines by inducing apoptosis. It was checked whether the apparent toxicity was related to the mechanism of action of these secretagogues and whether the toxicity was specific for B-cells. **Materials and Methods:** Secretagogue-exposed isolated mouse islets were examined by electron microscopy. The viability of secretagogue-exposed HIT cells was checked by MTT-testing. DNA fragmentation and DEVD-caspase activity in these cells were determined to distinguish between apoptosis and necrosis. **Results:** In islets exposed for 18 hr to a maximally effective concentration (100 μM) of idazoxan, phentolamine, alnidine, quinidine and tolbutamide (500 μM) there was an increased percentage of damaged B-cells but not other islet cell types. Depending on the secretagogue, 4 to 18% showed membrane ruptures and swelling of the mitochondria, whereas in control-incubated islets less than 2% were affected. To obtain a concentration-dependency of the secretagogue-associated toxicity HIT cells were exposed for 24 and 48 hr to 10, 100 and 1000 μM of the above compounds. High concentrations of idazoxan, phentolamine and quinidine led to a virtually complete block of MTT conversion whereas alnidine and tolbutamide were only moderately toxic. The reduction of MTT conversion in HIT cells correlated with the number of damaged B-cells. Only idazoxan and phentolamine at 100 μM caused DNA fragmentation and increased caspase activity, indicating apoptotic cell death. Diazoxide (300 μM) and D600 (50 μM) were practically ineffective to reduce the number of damaged B-cells in secretagogue-exposed islets, to prevent the decrease in MTT conversion or to diminish DNA fragmentation in HIT cells. **Conclusions:** B-cells are particularly susceptible to the toxicity of the secretagogues at the tested concentration. The toxicity varies widely between the secretagogues and involves both apoptosis and necrosis. Depolarization and calcium influx are not essential steps in the secretagogue-associated B-cell damage, thus the toxicity is not intrinsic to the insulinotropic mechanism of action.

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CELL SURFACE TRAFFICKING OF FAS IN PANCREATIC BETA-CELLS: DISSECTION OF FAS-EXPRESSION

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Background and Aims: Fas has been detected in a variety of organs including the liver, kidney, intestine, lung and ovary but not in the pancreatic islets of normal subjects. However, Fas has been identified exclusively in infiltrated islets of patients with recent-onset of Type-1 diabetes and the induction of Fas by cytokines released from islet infiltrating cells was postulated. The aim of the study is to investigate the relation of surface and intracellular Fas expression in beta cells exposed to cytokines.

Materials and Methods: Transformed beta cells (NIT-1) were cultured with IL-1b/IFN-g (100/1000 U/ml) for 6, 12, 24 and 48 h or kept untreated as controls (Co). Surface and intracellular Fas expression (% \pm SD) was analysed by flow-cytometry using the antibodies Jo2, AAP-221 and M-20 and confirmed by Western-blot analysis.

Results: Surface Fas expression was induced after 6h culture (IL-1b/IFN-g: 11.8 \pm 4.2%; Co: 1.8 \pm 2.6 %). Brefeldin A, a protein-secretion inhibitor, completely abolished the induction Fas (IL-1b/IFN-g: 0.6 \pm 0.9 %; Co: 0.6 \pm 0.79%). After 12h NIT-1 cells showed a constant level of surface Fas expression (12h: 13.1 \pm 3.8% vs. 0.7 \pm 1.1%; 24h: 15.0 \pm 6.9% vs. 2.1 \pm 2.2%; 48h: 15.3 \pm 6.2% vs. 0.5 \pm 0.7%). Intracellular Fas expression was comparable between cytokine-treated NIT-cells and controls as detected by AAP-221 (IL-1b/IFN-g: 72.2 \pm 7.7%; Co: 80.2 \pm 7.5%) and M-20 (IL-1b/IFN-g: 78.6 \pm 9.7%; Co: 81.0 \pm 11.0%). The independence of intracellular Fas expression from cytokine exposure was confirmed by Western-blot.

Conclusions: Beta cells of infiltrated islets seem to get sensitive to Fas-induced apoptosis by translocating the cysteine-rich domains to the cell surface. The results of the intracellular Fas staining indicate that parts of the transmembrane domain and/or intracellular death domain are expressed in beta cells independent of cytokine exposure.

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SERUM SOLUBLE FAS AND FAS LIGAND PROFILES IN AUTOIMMUNE DIABETES

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Background and Aims: Beta cell apoptosis has been associated with Type 1 diabetes onset. Fas and Fas ligand expression was detected in inflamed islets in pancreas sections of patients with recent-onset Type 1 diabetes mellitus. The aim of this study was to examine the balance between soluble Fas (sFas) and soluble Fas ligand (sFasL) and how that factors might reflect on etiopathogenesis of autoimmune diabetes.

Materials and Methods: The study included 20 recent-onset (RO; age 7.2 \pm 3.4 yrs, duration 0.4 \pm 0.2 mo), 20 long-standing Type 1 diabetic patients (LS; age 22.3 \pm 10.2 yrs, duration 9.0 \pm 4.5 yrs), 21 first-degree relatives (FDR; age 15.9 \pm 12 yrs) double positive for two disease-associated autoantibodies (ICA and GAD) and 19 age-matched healthy controls (HC). Circulating serum levels of sFas and sFasL were measured using a sandwich ELISA.

Results: In the LS group the level of sFas correlated with age ($p < 0.05$) and with the duration of the disease ($p < 0.05$). In the FDR group the level of sFasL inversely correlated only with age ($p < 0.0001$), while no correlation was found regarding ICA or GAD antibody titres. No such correlation was found in the RO group. The groups were then compared using the Mann-Whitney U test. The level of sFas in the RO group was higher than in the LS group of patients (12.5 \pm 3.9 vs. 11.0 \pm 4.8 U/ml, $p = N.S.$) but the difference was not significant. On the contrary the level of sFasL was higher in the RO group compared to LS group of patients (0.85 \pm 0.1 vs. 0.72 \pm 0.1 ng/ml, $p < 0.001$). Both sFas and sFasL were higher in RO patients compared to FDR subjects (12.5 \pm 3.9 vs. 8.4 \pm 4.3 U/ml, $p < 0.005$; 0.85 \pm 0.1 vs. 0.76 \pm 0.1 ng/ml, $p < 0.01$) and HC subjects (12.5 \pm 3.9 vs. 7.4 \pm 4.2 U/ml, $p < 0.005$; 0.85 \pm 0.1 vs. 0.7 \pm 0.1 ng/ml, $p < 0.005$). When LS group was compared to FDR and HC groups only sFas was higher in patients (11.0 \pm 4.8 vs. 8.4 \pm 4.3 U/ml, $p < 0.05$; 11.0 \pm 4.8 vs. 7.4 \pm 4.2 ng/ml, $p < 0.05$). Finally, although both sFas and sFasL were higher in the FDR group of subjects at risk compared to HC group, the difference was not significant (8.4 \pm 4.3 vs. 7.4 \pm 4.2 U/ml, $p = N.S.$; 0.76 \pm 0.1 vs. 0.7 \pm 0.1 ng/ml, $p = N.S.$).

Conclusions: Both sFas and sFasL are significantly elevated at the onset of the disease confirming the role of this apoptosis system in ongoing target tissue damage. After onset of the disease the level of sFasL decreases while sFas level remains relatively high. However, the relationship of sFas and sFasL with antibody markers of active autoimmunity was not confirmed.

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SURFACE EXPRESSION OF FAS AND ICAM-1 ON PANCREATIC ISLET BETA CELLS FROM DIABETES-PRONE BB RATS

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Background and Aims: Cytokines released by activated mononuclear cells suppress β -cell function and exhibit direct toxic effects on pancreatic islets. The aim of our study was to investigate cytokine-induced alterations of pancreatic islets from diabetes-prone BB rats and possible involvement of Fas in this context. **Materials and Methods:** Islets precultured for 3d were exposed to IL-1 β (10 U/ml) or IL-1 β (10 U/ml)+IFN- γ (500 U/ml)+TNF- α (500 U/ml) for 24h or not (control islets). After cytokine treatment islets were used for functional tests. Surface antigen expression was measured on single islet cells by FACS analysis using polyclonal antibody against Fas or monoclonal antibodies recognizing ICAM-1 (1A29) and β -cells (K14D10). Islet proteins were separated by SDS-PAGE followed by immunoblotting. **Results:** IL-1 β alone and in combination with IFN- γ + TNF- α decreased insulin content ($p < 0.001$) and insulin release to 20 mmol/l glucose ($p < 0.001$) compared to controls. Whereas no membrane alterations of IL-1 β treated islet cells were detectable combination of all 3 cytokines caused damage of cell membranes as measured by spontaneous 51 Cr-release (40.58 \pm 2.40% vs. 15.49 \pm 2.75% of controls) accompanied by impaired mitochondrial membrane potential and increased percentage of hypodiploid nuclei. All 3 cytokines together significantly diminished number of β -cells (87.4 \pm 2.6% vs. 95.6 \pm 0.7 % of controls) and increased ICAM-1 ($p < 0.001$) and Fas $^+$ β -cells (65.9 \pm 5.3 % vs. 33.8 \pm 4.6 % on IL-1 β treated islets vs. 3.8 \pm 0.9 % on controls) as well as ICAM-1 ($p < 0.001$) and Fas antigen density (10.72 \pm 0.54 logU vs. 8.77 \pm 0.82 log U on IL-1 β treated islets vs. 3.09 \pm 0.88 logU on controls). In protein extracts of cultured islets Fas was similarly detectable by immunoblotting in both cytokine-treated islets as well as in controls and in freshly isolated islets too. **Conclusions:** Our results suggest the possibility that pancreatic β -cells are destroyed by apoptotic mechanisms through upregulation of the "death receptor" Fas and that simultaneous activity of proinflammatory cytokines (IL-1 β , TNF- α) as well as type 1 cytokines (IFN- γ) enhance risk of Fas-mediated β -cell destruction.

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INHIBITION OF CYTOKINE-INDUCED FAS-EXPRESSION BY THE NO-SYNTHASE INHIBITOR AMINOGUANIDIN

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Background and Aims: Aminoguanidin (AG) is well known as a potent inhibitor of the inducible NO-synthase which was shown by inhibition of cytokine-induced NO-production by pancreatic islets and β -cell lines. Other NO-synthase inhibitors prevent cytokine-induced DNA cleavage and consequently apoptosis, which was correlated with NO production. We investigated whether AG is able to prevent cytokine-induced Fas-expression of the β -cell line RIN5AH. **Material and Methods:** RIN-cells (10^6 /ml RPMI 1640, 10% FCS) were cultured as follows: (1) in medium alone (control), (2) addition of IL-1 β (0.05 to 1.0 ng/ml) and IFN- γ (100 IU/ml), (3) addition of AG (5 mmol/l) and (4) addition of IL-1 β , IFN- γ and AG. After 48h NO-production was determined in the supernatant (Griess reaction, ELISA). The cells were harvested using trypsin and labelled for flow cytometry (FACS Calibur, BD) with the following monoclonal antibodies: OX18-PE (MHC I), 1A29-PE (ICAM-1), K14D10-FITC (β -cells) and anti-Fas (APO-1/CD95, detection with anti-rabbit-IgG-PE). **Results:** NO-production of control cells amounts to 0.5 \pm 0.2 nmol/ 10^6 cells ($n=9$) which is not influenced by AG alone (0.2 \pm 0.1 nmol/ 10^6 cells). NO is significantly induced by IL-1 β /IFN- γ and is dependent on IL-1 β concentration (119.3 \pm 31.3 nmol/ 10^6 cells at 0.05 ng/ml IL-1 β to 357.2 \pm 40.3 nmol/ 10^6 cells at 1.0 ng/ml IL-1 β , $p < 0.001$). AG inhibited NO-production completely (3.9 \pm 0.7 nmol/ 10^6 cells, $p < 0.001$). More than 96% of the control cells are positive for K14D10, 98.6 \pm 0.3% express MHC I and 77.6 \pm 2.3% express ICAM-1. Fas was measured on 18.9 \pm 2.5% ($n=16$) of control cells. IL-1 β /IFN- γ reduced the proportion of K14D10 $^+$ cells to 80%, enhanced 1A29 $^+$ cells to 89.6 \pm 1.7% ($p < 0.001$) and increased Fas-expression to 56.7 \pm 3.6% ($n=15$, 1.0 ng/ml IL-1 β , $p < 0.001$). Increase of Fas-expression was dependent on IL-1 β concentration (0.1 ng/ml: 37.0 \pm 6.0%; 0.05 ng/ml: 18.7 \pm 2.9%). AG inhibited the cytokine-triggered reduction of K14D10 $^+$ cells and reduced Fas-expression to control values at all IL-1 β concentrations (22.2 \pm 1.8%). **Conclusions:** Prevention of cytokine-induced NO-production by AG is accompanied by an inhibition of cytokine-induced Fas-expression and suggests a possible role of AG in the prevention of NO-mediated apoptosis.

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Evidence for a role of PKC delta in lipooapoptosis of beta-cells

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Background and Aims: Chronic incubation of isolated beta-cells of islets with FFAs impairs insulin secretion and induces apoptosis. The molecular mechanisms for these so called lipotoxic effects are poorly understood. Recent studies have shown that protein kinase C (PKC)-delta is activated during apoptosis. Since the PKC-delta represents the predominant PKC isoform being expressed in beta-cells we have studied whether this PKC is involved in lipooapoptosis of beta-cells.

Materials and Methods: The rat insulinoma cell line RIN1046-38 and primary beta-cells isolated from pancreas of mice were incubated with the unsaturated FFA palmitate. Intracellular distribution of PKC-delta was detected with an isoform specific antibody by confocal laser microscopy. Apoptosis was tested by DNA fragmentation assay.

Results: Palmitate (0.5 to 1 mM, 15 min.) induced a rapid translocation of PKC-delta from the cytosolic pool to the nuclear membrane both, in primary beta-cells as well as RIN1046-38 cells. Furthermore chronic incubation of RIN1046-38 cells with palmitate (>12 hours at 0.5 to 1 mM) resulted in a significant increase in apoptosis measured by DNA fragmentation which was 1.89 % in the basal condition and 7.2 % after palmitate incubation (n=3, % of total DNA). Coincubation of RIN1046-38 cells with rottlerin which specifically inhibits PKC-delta at the concentration we have used (5 µM) prevented palmitate induced apoptosis.

Conclusion: These data suggest a role of PKC-delta for palmitate induced beta-cell apoptosis. This mechanism may potentially be relevant for reduced beta-cell mass in obese Type 2 diabetic patients as well.

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Inducible expression of Bcl-2 using the Ru 486-regulated adenovirus vector system: effects on beta-cell viability and mitochondrial membrane potential

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Background and Aims: The beta-cells in the pancreatic islets of Langerhans are the targets of selective and progressive destruction in type 1 diabetes and beta-cell death is induced, at least partially, via apoptosis. Bcl-2 can inhibit apoptosis in pancreatic beta-cells suggesting its potential as a tool for gene therapy. Previous studies have shown that the protective effect of Bcl-2 may be correlated with preservation of mitochondrial membrane potential. Protonophores, such as carbonyl cyanide p-trifluoromethoxyhydrazone (FCCP), collapse the proton gradient across the mitochondrial inner membrane, resulting in the complete abolition of the mitochondrial membrane potential. The aim of this study was to obtain an efficient and inducible transduction system for expression of the antiapoptotic Bcl-2 gene in insulin producing cells as a potential tool for protecting against pancreatic beta-cell death in type 1 diabetes.

Materials and Methods: In this study we genetically engineered insulin producing cells with Bcl-2 gene using an adenovirus vector system based on the RU 486-regulated progesterone antagonist. Isolated pancreatic rat islet cells or rat insulinoma (RINm5F) cells were incubated with a mixture of pAdG5Bcl-2 adenovirus, expressing the Bcl-2 gene and pAdCMVProg helper virus at different viral concentrations (MOI) and different Ru 486 inducer concentrations. Bcl-2 overexpression was assessed by Western blot assay and the transfection efficiency was determined by FACS analysis. Bcl-2-expressing pancreatic beta-cells were treated with 5mM FCCP for 24 h and the viability of the cells was assessed using the colorimetric assay (XTT based) for cell viability. The mitochondrial membrane potential was assessed by using the lipophilic cationic membrane potential-sensitive dye JC-1.

Results: Beta-cells from dispersed pancreatic islets were transfected with an efficiency of more than 60% (at 30 MOI). The Bcl-2 protein expression levels could be adjusted by varying the amount of RU486 inducer reaching at most a 100-fold induction in RINm5F cells. Both JC-1-FACS analysis and XTT assays showed a significantly increased viability of Bcl-2-expressing beta-cells, which though, did not prevent mitochondrial membrane potential collapse following addition of FCCP.

Conclusions: The system permits an efficient and tight control of Bcl-2 gene expression in insulin producing cells, allowing further optimisations to an increased protection against beta-cell death in type 1 diabetes.

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Overexpression of Na/Ca exchanger increases apoptosis by inducing endoplasmic reticulum stress and activation of caspase-12 in insulin secreting cells.

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Background and Aims: In the pancreatic β-cell, Ca²⁺ regulates a large number of cellular processes, its concentration being in turn finely regulated by various channels, pumps and exchangers. In the β-cell, Ca²⁺ has also been implicated in triggering programmed cell death (apoptosis) and regulating death-specific enzymes. Therefore, the development of strategies to control Ca²⁺ homeostasis may represent a potential approach to prevent or enhance β-cell apoptosis. In the present study, we examined the effect of Na/Ca exchanger (isoform NCX1.7) overexpression on apoptosis in insulin-secreting cells (BRIN BD11 cells).

Materials and Methods: Rates of apoptosis were determined using the MTT assay, nuclear chromatin staining, quantification of DNA fragmentation and gel electrophoresis of low molecular weight DNA. Cytosolic and endoplasmic reticulum (ER) Ca²⁺ concentrations were measured using fura-2 and furaaptra, respectively.

Results: NCX1.7 overexpression significantly increased apoptosis in cells exposed (48h) to ER Ca²⁺-ATPase inhibitors, thapsigargin (2 µM) and cyclopiazonic acid (25 µM; P<0.001). No evidence of apoptosis was found after exposure to high concentrations of glucose (22.2 mM) or glibenclamide (10µM). NCX1.7 overexpression reduced the rise in cytosolic Ca²⁺ concentration induced by all agents and depleted ER Ca²⁺ stores. This depletion was attended by the activation of the ER-specific caspase (caspase 12), even in the absence of proapoptotic stimuli. Thapsigargin and ionomycin increased caspase 12 activation but to a larger extent in overexpressing than in control cells. Overexpression of NCX1.7 also sensitised the cells to Ca²⁺-independent proapoptotic signaling pathways, such as that mediated by nicotinamide (50 mM).

Conclusions: We show that Na/Ca exchanger overexpression, by depleting ER Ca²⁺ stores, induces ER stress with resulting activation of caspase 12, and increase in apoptotic cell death by Ca²⁺-dependent and independent pathways.

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Differential effects of genistein on NaF-induced apoptosis in primary islet cells vs RINm5F cells

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Background and Aims: Previous studies have revealed that both normal pancreatic islets and clonal RINm5F-cells can be induced to undergo apoptosis upon incubation with NaF and that this response is enhanced in cells treated with pertussis toxin. On this basis, it has been assumed that RINm5F cells represent a suitable model for study of the signalling mechanisms involved in the regulation of islet apoptosis by G-protein dependent pathways. In the present work, we have investigated this proposition further and show that the response of pertussis toxin-treated human and rat islets differs markedly from that of RINm5F cells, when the effects of the tyrosine kinase inhibitor, genistein, are studied.

Materials and Methods: Annexin-V was combined with carboxyfluorescein staining and fluorescence microscopy to identify viable, necrotic and apoptotic cells in isolated human and rat islets and in populations of cultured RINm5F cells.

Results: Treatment of either normal islets or RINm5F cells with 5mM NaF resulted in a marked increase in apoptotic cells (from 0.5±0.1% to 21±2% in rat islets; 3±0.5% to 11±1% in human islets and 1±0.1% to 12±2% in RINm5F cells; p<0.001 in each case). Addition of 100µM genistein alone, also increased apoptosis in each preparation (rat islets: 23±3%; human islets: 17±2%; RINm5F cells: 7±2%; p<0.001). However, the combination of genistein and NaF dramatically reduced the overall extent of apoptosis in RINm5F cells (to control levels; p<0.001) while it provoked a further increase in apoptosis in rat (to 32±3%; p<0.01) and human (to 22±2%; p<0.01) islets. This difference could not be accounted for by any corresponding alteration in the numbers of necrotic cells in the various preparations, suggesting that the response of normal islets to genistein is quite different from that seen in RINm5F cells. In support of this, whereas the ability of pertussis toxin to cause an enhancement of NaF-induced apoptosis was antagonised by genistein in RINm5F cells (from 26±3% to 6±1%; p<0.001) it was further increased in primary islets (from 14±2% to 22±4%; p<0.01).

Conclusions: These results indicate that there are marked differences in the apoptotic responses of RINm5F cells to stimuli controlling G-protein-dependent mechanisms, by comparison with normal rat and human islets. They suggest that caution should be exercised when extrapolating conclusions about the regulation of apoptosis in cultured beta-cell lines to primary islet cells.

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APOPTOSIS AND IL-1BETA mRNA EXPRESSION IN SYNGENEIC RAT ISLET GRAFTS

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Background: transplanted islets are particularly vulnerable in the first days after transplantation (Tx) and it is essential to know and identify the mechanisms of beta cell destruction and protection.

Aims: to determine in syngeneic rat islet Tx whether: 1) beta cell apoptosis is increased in the first days after Tx, 2) cytokine genes are expressed in islet grafts, 3) insulin treatment has a beneficial effect in the outcome of the graft. Material and

Methods: two groups of streptozotocin diabetic Lewis rats were Tx with 500 syngeneic islets, an insufficient beta cell mass to achieve normoglycaemia. One group was treated with insulin from day 7 before Tx to day 14 after Tx, and the other group was not treated with insulin. Grafts were harvested 1, 3 and 7 days after Tx. Apoptosis was determined by TUNEL technique and expressed as percentage of positive beta cells. The proinflammatory cytokine IL-1beta mRNA expression was measured by semi-quantitative RT-PCR and indexed to a housekeeping gene (cyclophilin) expression.

Results: in insulin-treated group, when insulin was discontinued rats remained normoglycaemic despite the Tx of an insufficient beta cell mass. As expected, the non-insulin treated group remained hyperglycaemic throughout the study. Beta cell apoptosis was increased on day three after Tx ($0.34 \pm 0.11\%$) compared to apoptosis in pancreas of control animals ($0.05 \pm 0.02\%$). The expression of proinflammatory IL-1beta cytokine transcripts was increased in islet grafts on day 1 and 3 after Tx.

Conclusions: beta cell apoptosis was increased in the first days after syngeneic rat islet Tx. The expression of IL-1beta in these syngeneic islet grafts initially after Tx suggests that proinflammatory cytokines may play a role in beta cell apoptosis. Normoglycaemia improved the prognosis of islet Tx in rats; we are currently studying whether there are any differences in the expression of cytokines between hyperglycaemic and normoglycaemic recipients.

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HUMAN BETA CELLS ARE HIGHLY RESISTANT TO STREPTOZOTOCIN IN VIVO.

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Background and Aims: Streptozotocin (STZ) is the prototype for beta cell death via DNA damage precipitating poly-ADP-ribose synthetase activation followed by lethal NAD depletion, a mechanism well-documented in rodents. It is unclear whether human islets are susceptible to this type of damage. For obvious reasons, no one has ever performed a dose response study on humans. Our aim is to study human islets in vivo.

Materials and Methods: Islets were isolated from CD1 mice and Lewis rats (both sensitive to STZ), tilapia fish (highly resistant), and from man (supplied by JDFI Human Islet Distribution program) and were transplanted under the renal capsules of STZ-diabetic (non-fasting blood glucose > 400 mg/dl \times 1 week) athymic nude mice. Islet recipients were then followed for 30 days (blood glucose measurements 3x/week). Recipients that were uniformly normoglycemic (blood glucose < 200 mg/dl) and had normal glucose tolerance tests (GTTs) on day 30 were randomly assigned into treatment groups. We then injected the groups of recipient mice with increasing intravenous (IV) doses of STZ, followed their blood glucose levels for one week, and repeated the GTTs on any mice that were not overtly diabetic. Next, the mice were killed and graft-bearing kidneys and native pancreata processed for histology (including insulin stains).

Results: Based upon 3 criteria (i.e., blood glucose levels, GTTs, and islet histology), the following observations were made: (1) Recipients of rat islets were uniformly resistant to 25 mg/kg STZ (n=3) (i.e., by all 3 criteria) but were uniformly diabetic at doses of either 50 (n=3) or 75 (n=3) mg/kg IV. (2) Recipients of CD1 mouse islets were resistant to 75 mg/kg (n=3) but were uniformly diabetic with doses of either 150 (n=3) or 200 (n=2) mg/kg IV. (3) Recipients of the tilapia islets were uniformly resistant to doses of 300 (n=3) and 400 (n=3) mg/kg STZ; however, at a dose of 450 mg/kg IV (n=4), 2 mice were non-diabetic, 1 had an abnormal GTT, and 1 died while normoglycemic. (4) Recipients of human islets were resistant to 100 (n=1), 200 (n=1), 300 (n=2), 400 (n=3), and 450 (n=1) mg/kg IV. Doses higher than 450 mg/kg IV were not tested because of the finite solubility of STZ in citrate buffer and the desire to avoid marked hemodilution by injecting excessive volume.

Conclusions: The results of STZ dose-response profiles in recipient nude mice bearing long-term Lewis rat, CD1 mouse, or tilapia islets were the same as results of published dose-response data for each donor species. Therefore, we extrapolate that our results based on human islet grafts in murine recipients closely reflects STZ sensitivity in man. We conclude that human islets are highly resistant to STZ in vivo.

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Nitric Oxide and Free Radicals in Beta-Cell Destruction

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INTERLEUKIN-1B DECREASES THE RESISTANCE OF RAT B-CELLS TO DIFFERENT AGENTS.

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The cytokine, interleukin-1 β (IL-1 β) has been implicated to play an important role in the β -cell damage of type 1 diabetes. Recent work has highlighted the importance of Fas expression is increased on β -cells, in response to IL-1 β treatment. Here, we report that IL-1 alone induces apoptosis in rat islets and we confirm, that IL-1 further sensitises rat β -cell to the induction of apoptosis by a Fas-dependent mechanism in case of subsequent influence of streptozotocin or IL-1, but not alloxan.

Isolated rat pancreatic islets were precultured for 6d and then exposed to IL-1 β (10 U/ml) for 18h or remained untreated (control). Every other day (by 6th d) after cytokine treatment, cell viability was measured in colorimetry tests with neutral red. CD95 antigen was measured by FACS analysis on prepared with EDTA single islet cells. On the fourth day after the moment of cytokine treatment both experimental groups of pancreatic islet cells were again treated by IL-1 β (10U/ml) for 18h, as well as by streptozotocin (0,1mM) or alloxan (1,0 mM) for 0,5h. Part of the cells in these experimental groups was left without repeated influence. The CD95 antigen expression was observed in 17 \pm 2,1% for interleukin treating cells, but in four days its content was 5 \pm 0,1%. The repeated addition of IL-1 β or its combination with streptozotocin caused a reduction of β -cell number ($p<0,05$) (23 \pm 4,1% vs intact cells culture and 40 \pm 6,3% vs once treated with interleukine cells culture). CD95 antigen expression increased to 56 \pm 8% in these β -cells. Similar changes were observed when streptozotocin was used on the fourth day after initial influence of IL-1 β . But only 20 \pm 2% cells expressed CD95 antigen in this case. The same regime incubation with alloxan didn't demonstrate reliable difference of CD95 antigen expression vs control. But in this case observed reduction of β -cells number 26 \pm 5,6% vs once treated with interleukine cells culture. It is possible to conclude that interleukin-1 β is not only cytotoxic for β -cells, but make them more sensitive to cytotoxic agents such as streptozotocin, alloxan and also interleukin-1 β itself. This cytokine-induced decrease of β -cell stability is associated with changes in CD95 antigen expression. But in case of alloxan it was realized by quite different mechanisms of β -cells destruction.

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QUANTIFICATION OF THE CONTRIBUTION OF PEROXYNITRITE TO MIXED RADICAL- AND NITRIC OXIDE-MEDIATED BETA CELL DEATH
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Background and Aims: The mixed radical donor, SIN-1, and the pure nitric oxide (NO) donor, S-nitrosoglutathione (SNOG) are widely used to investigate the role of reactive species in beta cell cytotoxicity. However, the relative contribution of the reactive species generated by these compounds is little understood. We have quantified the levels of peroxynitrite (PN) generated by the radical donors using DCDHF oxidation and have used the specific PN scavenger, FeTPPS, to investigate the contribution of PN to SIN-1 and SNOG-mediated cytotoxicity.

Materials and Methods: RINm5F cells were treated for 24 hours with 500 μ M SIN-1 (or 500 μ M SNOG) with and without 20 μ M or 40 μ M FeTPPS. The effects on cell viability were assessed by Trypan blue exclusion cell counts on detached cells and by acridine orange staining and fluorescence microscopy. Formation of DCDHF's oxidation product, DHF, was measured by spectrophotometric analysis at 500nm.

Results: 20 μ M and 40 μ M FeTPPS completely abolished the cytotoxic effects of 500 μ M SIN-1 (Mean dead cells/ml \pm SEM (n=8): Control: 6906 \pm 425; +500 μ M SIN-1: 25500 \pm 1025*; +500 μ M SIN-1+20 μ M FeTPPS: 7813 \pm 427*; + 500 μ M SIN-1+ 40 μ M FeTPPS: 6563 \pm 319**). * p<0.001 vs. control; ** p<0.001 vs. 500 μ M SIN-1). 20 μ M and 40 μ M FeTPPS also significantly reduced the cytotoxic effect of SNOG, although to a much lesser extent than that seen with SIN-1 (Mean dead cells/ml \pm SEM (n=8):

Control: 8219 \pm 238; +500 μ M SNOG: 24938 \pm 1326*; +500 μ M SNOG + 20 μ M FeTPPS: 20000 \pm 1502*; +500 μ M SNOG + 40 μ M FeTPPS: 18188 \pm 1250**). *p<0.001 vs. control; **p<0.01 vs. 500 μ M SNOG). The mixed radical donor, SIN-1 was found to generate large amounts of PN in total (in excess of 25 μ M generated from 100 μ M SIN-1 in 2hour 30 min). More surprisingly, the pure NO donor, SNOG also led to significant amounts of PN being formed, although over a slower time course (20 μ M from 100 μ M SNOG in 7 hours). **Conclusions:** The relative difference in the effects of PN-scavenging by FeTPPS on SIN-1- and SNOG-mediated cytotoxicity could therefore reflect differences in their dynamics of PN generation or, equally, the relative contribution of NO and PN in mediating cell death. These results demonstrate the potential of DCDHF and FeTPPS as tools in dissecting the roles of specific reactive species in beta cell death.

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REDUCED INDUCTION OF PROSTAGLANDIN E₂ FROM CYTOKINE TREATED β -CELLS DEFICIENT IN INDUCIBLE NITRIC OXIDE SYNTHASE
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Background and Aims: In rodent islets, exposure to proinflammatory cytokines including interleukin-1 β (IL-1 β) and interferon- γ (IFN- γ) induces expression of inducible nitric oxide synthase (iNOS) and subsequent nitric oxide (NO) formation. NO may suppress islet function, and the use of iNOS inhibitors or a deletion of the iNOS gene has been shown to be protective both *in vitro* and in animal models of type 1 diabetes. However, we and others have shown that cytokines may induce NO-independent suppression of islet function. In parallel to iNOS induction and NO formation, IL-1 β induces cyclooxygenase-2 (COX-2) expression and prostaglandin E₂ (PGE₂) formation. PGE₂ is suggested to impair glucose stimulated insulin release. COX-2 expression is activated by similar signalling pathways as iNOS, and NO may affect COX-2 activity. The aim of the present study was to examine if iNOS deficiency affects the PGE₂ formation from cytokine treated mouse islets. **Materials and Methods:** Islets isolated from iNOS^{-/-} and wild-type (wt) mice were cultured for 6-7 days and then exposed to IL-1 β (25 U/ml) or a combination of IL-1 β (25 U/ml) and IFN- γ (1000 U/ml) for 48 h. PGE₂ content in culture medium was measured by ELISA, and NO formation was quantified using the Griess reagent. **Results:** IL-1 β alone induced a significant increase in NO formation from wt islets, which was potentiated by IFN- γ . Cytokine exposure to iNOS^{-/-} islets did not induce NO formation. In parallel to NO formation, IL-1 β and IL-1 β +IFN- γ induced a significant increase in PGE₂ formation from wt islets. In iNOS^{-/-} islets, IL-1 β and IL-1 β +IFN- γ induced PGE₂ formation, although this increase was about 80% lower compared to wt islets. Wt islets: control 0.17 ± 0.13 ; IL-1 β $0.89 \pm 0.28^*$; IL-1 β +IFN- γ $1.24 \pm 0.49^*$ ng PGE₂/100 islets * 48 h, n=5; iNOS^{-/-} islets: control 0.04 ± 0.03 ; IL-1 β $0.19 \pm 0.05^*$; IL-1 β +IFN- γ $0.20 \pm 0.04^*$ ng PGE₂/100 islets * 48 h, n=5. * $p < 0.05$ vs. control (within the same strain), using ANOVA. **Conclusions:** We conclude that in iNOS deficient mouse islets, IL-1 β or IL-1 β + IFN- γ have a reduced ability to induce PGE₂ formation.

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EFFICACY OF 1400W, A NOVEL INHIBITOR OF INDUCIBLE NITRIC OXIDE SYNTHASE, IN PREVENTING RODENT PANCREATIC ISLET DAMAGE

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Background and Aims: Nitric oxide (NO), produced by the enzyme inducible nitric oxide synthase (iNOS), has been implicated as a mediator of destruction of β -cells in type 1 diabetes. *In vivo* studies suggest that inhibition of NO formation might protect against diabetes in animal models. However, inhibitors tested so far have not shown a desired selectivity against iNOS. Our aim is to test if 1400W (N-(3-(Aminomethyl)benzyl)acetamidine), a new and selective inhibitor of iNOS can prevent interleukin-1 β (IL-1 β) induced suppression of pancreatic islet function *in vitro* and multiple low-dose streptozotocin (MLDS) induced diabetes *in vivo*. **Materials and Methods:** We exposed precultured rat pancreatic islets for 48 h to medium with or without 1, 10 and 50 μ M 1400W and in the presence or absence of 25 U/ml IL-1 β . We did two *in vivo* experiments in which male C57BL/Ks mice received five daily i.p. injections with streptozotocin (STZ) while the inhibitor were given i.p. for ten consecutive days (8.34 mg/kg body weight, once a day, in the first experiment and 20 mg/kg body weight, twice a day, in the second). Statistical analysis was done by ANOVA and data presented as means \pm SEM. **Results:** The inhibitor alone did not affect any of the islet functions analyzed *in vitro*. However, at 50 μ M, it fully counteracted both the suppression of glucose oxidation rate (control 437 ± 30 , IL-1 β $252 \pm 47^*$, IL-1 β + 1400W $441 \pm 23^{\#}$ (pmol/10 islets * 90 min.), n=4, * $p < 0.01$ vs. control and $\#p < 0.01$ vs. IL-1 β) and the increase in NO production (control 5.2 ± 3.1 , IL-1 β $10.7 \pm 6.4^*$, IL-1 β + 1400W $6.0 \pm 2.9^{\#}$ (pmol/10 islets * h), n=5, * $p < 0.001$ vs. control and $\#p < 0.001$ vs. IL-1 β) caused by IL-1 β . The marked decrease, seen with IL-1 β , in glucose stimulated insulin release was also counteracted by the addition of 1400W (50 μ M). In this case from ~14 % of the control value to ~54 % (control 30.0 ± 2.4 , IL-1 β $4.18 \pm 1.2^*$, IL-1 β + 1400W $16.3 \pm 3.0^{\#}$ (ng/10 islets * h), n=5, * $p < 0.001$ vs. control, $\#p < 0.01$ vs. control and $\#p < 0.05$ vs. IL-1 β). Mice treated *in vivo* with STZ gradually developed hyperglycaemia and none of the concentrations of 1400W used was able to prevent this. **Conclusion:** 1400W could counteract the IL-1 β induced suppression of rat pancreatic islets *in vitro*, but failed to protect against MLDS induced diabetes *in vivo*, at least with the administration protocols used here in. The latter failure may be due to a too short duration of 1400W *in vivo*.

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Inhibition of nitric oxide synthase prevents Ca²⁺-dependent apoptosis of pancreatic beta-cells in the calmodulin-overexpressing transgenic mice.

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Background and Aims: Beta cell-specific overexpression of calmodulin in transgenic mice results in a sudden-onset of hyperglycemia associated with rapid disappearance of beta cells. We investigated the mechanisms underlying the beta cell loss.

Materials and Methods: Pancreata from nontransgenic or transgenic mice were examined by light and electron microscopic approaches. Beta cell apoptosis was detected by either the modified terminal deoxynucleotidyl transferase mediated deoxyuridine triphosphate nick-end labelling (TUNEL) method or electron microscopy.

Results: Ultrastructural analysis of pancreata from the transgenic mice demonstrated decreased size of beta-cells coupled and number of the insulin granules. Transgenic beta cells appeared apoptotic but without necrotic changes of the cells or infiltration of lymphocytes. Injection of tolbutamide into the transgenic mice increased the number of TUNEL-positive beta cells. Hyperglycemia in the transgenic mice was prevented by injection of the nitric oxide-synthase (NOS) inhibitor, Nw-nitro-L-arginine methyl ester (L-NAME) and beta cell size and granule number were retained. Immunofluorescent staining demonstrated preferential distribution of neural nitric oxide synthase (nNOS) in pancreatic beta cells.

Conclusions: These results suggest Ca²⁺/calmodulin-dependent NO-production by nNOS may be involved in the generation of apoptosis in beta-cells of calmodulin-overexpressing transgenic mice.

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2(S)-amino-6-borohexanoic acid, an arginase inhibitor, increases cytokine-induced nitric oxide generation in rat islets of Langerhans.

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Background and Aims: Arginine-derived nitric oxide (NO) produced in response to pro-inflammatory cytokines is thought to play an important role in beta cell destruction. We have shown previously that islets express high levels of both isoforms of the arginine-utilising arginases. While it is possible that NO generation can be restricted by a high rate of flux of arginine through arginase, there is no previous evidence from islet studies to support this notion. Our aim was to assess the effect of the specific arginase inhibitor 2(S)-amino-6-borohexanoic acid (ABH) on rat islet arginase activity and to investigate the effect of arginase inhibition on the rate of cytokine-induced NO generation in islets.

Materials and Methods: Cytosolic extracts were prepared from freshly isolated adult male Wistar islets. Arginase activity in supernatants was measured by the conversion of 14C-guanidino-arginine to 14CO₂. For NO generation studies, islets were cultured in RPMI 1640 containing 1.1mM arginine for 48h followed by 24h treatment with 20U/ml r human IL-1 β in medium containing 1.1mM, 0.1mM or 0.01mM arginine. Nitrite in the culture medium was measured using the Griess assay.

Results: Addition of 50uM ABH to islet extracts significantly reduced arginase activity: control 96.8 ± 12.4 mU/mg protein, + ABH 27.4 ± 2.7 mU/mg (mean \pm SEM, n=3, $P < 0.006$). In culture experiments, ABH significantly increased cytokine-induced NO generation by islets cultured in medium containing 0.01mM arginine (IL-1 alone 37.7 ± 9.5 pmol/ug protein, IL-1 + ABH 64.6 ± 9.2 pmol/ug, n=5, $p < 0.01$). Culture of islets in medium containing 0.1mM arginine enhanced cytokine-induced nitrite generation and this was also significantly increased by treatment with ABH (IL-1 alone 63.6 ± 7.5 pmol/ug protein, IL-1 + ABH 79.6 ± 4.8 pmol/ug, n=5, $p < 0.03$). At 1.1mM arginine, ABH did not significantly affect cytokine-induced nitrite generation (IL-1 alone 69.8 ± 6.5 pmol/ug protein, IL-1 + ABH 84.5 ± 12.7 pmol/ug, n=3).

Conclusions: These results are consistent with an increased activity of islet nitric oxide synthase due to an increased availability of a rate-limiting substrate following inhibition of arginase by ABH. This effect was dependent on the concentration of arginine in the culture medium. Genetic, physiological and pharmacological factors that affect islet arginase activity may therefore be of importance to the control of NO generation and subsequent beta cell damage during insulinitis.

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CATALASE AND SUPEROXIDE DISMUTASE ACTIVITIES IN DIABETES-PRONE BB/S RAT ISLETS

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Background and Aims: It has been proposed that low activity of antioxidant enzymes, including superoxide dismutase and catalase in pancreatic beta cells may increase susceptibility to autoimmune driven free-radical damage. Previous measurements of islet superoxide dismutase are inconsistent and catalase has not been estimated in an animal model of autoimmune diabetes. Our aim was to compare antioxidant enzyme activities in islets from pre-diabetic diabetes-prone BB (BB DP), diabetes resistant (BB DR) and Wistar control rats.

Materials and Methods: The BB/S colony of rats consists of diabetes prone (BB DP, incidence 80%, onset 70-90d) and diabetes resistant (BB DR, diabetes-free > 17 generations). Sonicated extracts were made from freshly isolated islets for determination of activities of catalase, by conversion of methanol and hydrogen peroxide into water and formaldehyde (540nm), and superoxide dismutase measured by its inhibition of the chemiluminescence of luminol. Data are expressed as mean \pm SEM, statistical comparison by Student's t-test.

Results: Freshly isolated islets from 52d old BB DP rats had lower superoxide dismutase activity compared to age-matched BB DR rats (1.1 ± 0.26 vs 4.48 ± 1.1) mU/ug protein, $P < 0.05$, $n = 7-8$.

Islets from 55d old BB DP rats had significantly lower catalase activity than islets from age-matched Wistar rats (0.10 ± 0.02 vs 0.31 ± 0.04) μ kat/ug protein in extract, $P < 0.01$, $n = 4-6$. There was no difference in percentage insulin secretion between the groups, however BB DP islets contained and secreted less insulin than Wistar islets. Treatment of normal cultured Wistar islets and RINm5F cells with cytokines (IL-1, IFN- γ , TNF- α) or nitric oxide both decreased catalase activity to 70% and 50% of that in untreated islets and cells.

Conclusions: Islets from diabetes-prone BB rats contain less catalase and superoxide dismutase activity compared to islets from BB diabetes-resistant and Wistar rats. This may be the result of altered gene expression of either antioxidant enzyme or of increased cytokine activity leading to nitric oxide-induced inhibition of catalase activity.

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HYDROGEN PEROXIDE-BASED SELECTION OF RINm CELLS IMPROVES CELL RESISTANCE TO OXIDATIVE STRESS.

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Background and Aims: Beta-cell lines constitute a potential source for future transplantation of engineered, insulin-producing cells. The cell defense system is believed to be crucial for post-transplantation long term success. Following our recent findings on selection of RINm cells for STZ and alloxan resistance (*JEDR*, 1: 2000), we further analyzed the effect of hydrogen peroxide (HP)-based selection on RINm cell defense mechanisms against oxidative stress. **Materials and Methods:** Resistance to HP (RINmHP) was obtained by repeated exposure of parental RINm cells to 100 μ M and 200 μ M of HP. Cell viability was estimated by the MTT colorimetric method, while defense potential was estimated by determination of catalase activity and hydrogen peroxide degradation in the cultured cells. Cell functional capacity was evaluated by insulin response to glucose and IBMX, and by determination of cell insulin content. **Results:** We found that RINmHP resistance to 200 μ M and 300 μ M of HP was 3.5 and 5.8-fold higher when compared to that of parental RINm cells ($p < 0.01$). In addition, the half-life of HP in the RINmHP culture medium was lower than that of parental cells. These data correspond with the 2-fold higher activity of catalase in RINmHP compared to that obtained in parental cells. The better defense properties of the selected cells were not found to be associated with any significant changes in the cell's capacity to respond to stimulation. **Conclusion:** HP treatment can be considered as a method to obtain insulin-producing cells which are resistant to oxidative stress due to their higher level of catalase activity.

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JNK BUT NOT MEK AND p38 INHIBITION REDUCES IL-1 β INDUCED RIN CELL DEATH INDEPENDENTLY OF NO PRODUCTION.

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The aim was to investigate the effects of a JNK inhibitor versus the effects of the MEK and p38 inhibitors on IL-1 β induced cell death and NO production in rat insulinoma (RIN) cells. **Background:** IL-1 induced NO production and cell death is decreased by inhibitors of the mitogen activated protein (MAP) kinases ERK and p38 in rat islets of Langerhans. However, it has been suggested that IL-1 induced cell death in cell lines is preferably signalled via the MAP kinase JNK. **Methods:** RIN-5AH-T2B cells (250,000 cells/mL) were seeded in RPMI+10% FCS and precultured for 24 hours. One hour prior to IL-1 exposure the cells were incubated with 10 μ M p38 inhibitor SB203580 (p38i) and 100 μ M MEK inhibitor PD098059 (MEKi) or 1 μ M JNK inhibitor tat-JBD (JNKi). Cells were exposed to 400-600 U/mL rhIL-1 β for 24 hours (using MEKi and p38i) or 48 hours (using JNKi). NO production was measured by Griess reaction, whereas cell death was measured by Propidium Iodide (PI) or Hoechst/PI staining. **Results:** NO production was increased significantly after exposure to IL-1 ($P < 0.01$). MEKi and p38i significantly reduced IL-1 induced NO production with 50% ($P < 0.05$) whereas JNKi had no effect on IL-1 induced NO production. IL-1 induced cell death ($P < 0.05$ versus control) was increased by 100% by MEKi and p38i ($P < 0.05$), whereas JNKi reduced IL-1 induced cell death by 50% ($P < 0.001$). **Conclusion:** We suggest that IL-1 induced cell death in RIN cells is induced by a high JNK activity relative to a low ERK and p38 activity, and further that JNK signalling leads to cell death independently of NO production.

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COMPARISON OF THE FUNCTIONAL PROPERTIES OF ISLETS FROM PREDIABETIC AND DIABETIC *PSAMOMMYS OBESUS*

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Background-Aim: When fed on a high energy (HE) diet, *P. obesus* rapidly develops type 2 diabetes. Within 5 days of hyperglycemia, glucose-stimulated insulin secretion (GSIS) is largely reduced, likely as a result of β cell degranulation. However, little is known about β cell stimulus-secretion coupling in *P. obesus*. **Methods:** Islets isolated from 12 prediabetic (low-energy LE diet) and 14 hyperglycemic (5 days HE diet) *P. obesus* (blood glucose = 4.4 ± 0.2 and 19 ± 1 mmol/l, respectively) were cultured overnight in either 5 mmol/l glucose (G5) for LE islets or G10 for HE islets. Islet NAD(P)H autofluorescence and cytosolic calcium concentration ($[Ca^{2+}]_i$) were measured during perfusion with G0.5, 2, 4, 6, and 10, whereas insulin secretion was assessed at G2, 6 and 10. **Results:** In LE islets, glucose dose-dependently increased NAD(P)H with threshold, half-maximal and maximal effective concentrations of 2, 5.1 and 10 mmol/l. Corresponding values for glucose increase of $[Ca^{2+}]_i$ were 4, 5.6 and 10 mmol/l. These metabolic and $[Ca^{2+}]_i$ changes resulted in a dose-dependent GSIS (~10 and 20 fold increase over G2 in G6 and G10 respectively). In comparison, islets from HE animals had a significantly higher sensitivity to glucose for NAD(P)H and $[Ca^{2+}]_i$ responses, with threshold, half-maximal and maximal effective concentrations (mmol/l) of 2, 3.3 and 6 for NAD(P)H and 2, 2.7 and 6 for $[Ca^{2+}]_i$, respectively. Strikingly, $[Ca^{2+}]_i$ was already found to oscillate in G2, resulting in a 18-fold higher rate of secretion compared to LE islets, despite a 4-fold reduction in insulin content. However, raising glucose to 10 mmol/l did not further stimulate insulin secretion. A similar shift in glucose sensitivity was observed in freshly isolated islets. **Conclusion:** Our results suggest that the *in vivo* defect of GSIS in HE *P. obesus* results from the complex interplay between decreased maximal secretory output due to β cell degranulation and increased glucose sensitivity as indicated by maximal NAD(P)H and $[Ca^{2+}]_i$ levels at low physiological glucose concentration.

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DISORGANIZATION OF CYTOPLASMIC Ca^{2+} OSCILLATIONS AND PULSATILE INSULIN SECRETION IN ISLETS FROM *ob/ob* MICE

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In normal mouse islets, glucose induces synchronous cytoplasmic $[Ca^{2+}]_i$ oscillations in all β -cells, and each oscillation triggers a pulse of insulin secretion. Chronically hyperglycemic and hyperinsulinemic *ob/ob* mice were used to evaluate the impact of an overstimulation of insulin secretion on this fine regulation of islet function. **Methods:** Islets were isolated from 8-12 m-old *ob/ob* mice and their age-matched lean littermates. After 18h culture in 10 mmol/l glucose, islet $[Ca^{2+}]_i$ was measured (fura PE3 technique), sometimes simultaneously with insulin secretion. **Results:** During stimulation with 9-12 mmol/l glucose, a lack of synchronization between $[Ca^{2+}]_i$ oscillations was observed in only 6% of lean islets vs 65% of *ob/ob* islets. Culture of *ob/ob* islets in 5.5 mmol/l glucose increased the incidence of desynchronization. In some islets the desynchronization was subtle (missing oscillation or variable shift between oscillations in different regions). In other islets, oscillations with distinct periods and shapes continuously occurred in different regions. Sometimes, the whole islet was crossed by $[Ca^{2+}]_i$ waves with variable starting points. Only small (40% of the volume of the others) *ob/ob* islets were synchronized, but there was a large overlap between sizes of well synchronized lean islets and desynchronized *ob/ob* islets. In lean and *ob/ob* islets stimulated with 12 mmol/l glucose, regular and synchronous $[Ca^{2+}]_i$ oscillations were accompanied by parallel oscillations of insulin secretion. In poorly synchronized *ob/ob* islets, the pattern of secretion was also irregular but followed the pattern of the global $[Ca^{2+}]_i$ change in the whole islet. **Conclusions:** Hyperstimulation of β -cells and/or hyperplasia of the islets disrupt the regularity of $[Ca^{2+}]_i$ oscillations and associated pulsatility of insulin secretion. These observations made in *ob/ob* mouse islets suggest a mechanism for the irregularity of insulin oscillations in type 2 diabetic patients.

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Defective glucose-regulated insulin gene expression limits insulin production in the *Psammomys obesus* model of type 2 diabetes.

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Background and Aims: *Psammomys obesus* (*P. obesus*), an animal model of type 2 diabetes, shows a marked depletion of insulin content in response to increased secretory demand. We have previously shown that *P. obesus* islets fail to increase insulin gene expression in response to glucose, probably due to the absence of the conserved form of the transcription factor PDX-1. In the present study we have evaluated the interrelationship between insulin gene expression, proinsulin biosynthesis and immunoreactive insulin (IRI) content in islets from the diabetes-prone *P. obesus* and from the diabetes-resistant Sprague-Dawley (SD) rat.

Materials and Methods: Islets of *P. obesus* and SD rats were cultured at various glucose concentrations (1.7 to 16.7 mmol/l) for 3, 6 and 24 h and then analyzed for insulin mRNA by quantitative RT-PCR, proinsulin biosynthesis by leucine incorporation into proinsulin and IRI content and secretion by RIA.

Results: Insulin mRNA content was 2-3 fold higher in *P. obesus* islets cultured in 3.3 mmol/l glucose compared to islets in 1.7 mmol/l glucose; no further increase was observed at higher glucose concentrations. The failure to increase insulin mRNA in response to glucose concentrations higher than 3.3 mmol/l was associated with a blunted increase in proinsulin biosynthesis (~1.5 fold). In contrast, rat insulin 1 mRNA was augmented in a dose-dependent manner by a wide range of glucose concentrations (~3 fold increase at 3.3 relative to 1.7 mmol/l glucose and 2-4 fold at 16.7 relative to 3.3 mmol/l glucose). This was accompanied by a marked increase in proinsulin biosynthesis (6 fold increase at 3.3 vs. 1.7 mmol/l glucose and approximately 9 fold increase at 16.7 vs. 3.3 mmol/l glucose). Despite the 2-3 fold higher insulin secretion at 16.7 mmol/l glucose in rat compared to *P. obesus* islets, insulin content was significantly lower in *P. obesus* islets.

Conclusions: The attenuated insulin gene expression in response to glucose stimulation in *P. obesus* is associated with a marked reduction in glucose-dependent proinsulin production. These findings support the hypothesis that inadequate regulation of insulin gene expression by elevated glucose contributes to the failure of *P. obesus* to cope with increased secretory demand with depleted insulin stores and diabetes as a consequence.

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Rescue of beta-cell exhaustion after the development of diabetes mellitus in db/db mice

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Background and Aims: It has been shown that poor glycemic control results pancreatic beta-cell damages. In this study, we demonstrated the possibility to rescue an exhaustion of beta cells by reducing stresses of insulin secretion using diazoxide, ATP sensitive K-channel opener, and pioglitazone.

Materials and Methods: 4 groups of db/db mice (n=6 per group): i) control db/db mice (C), ii) db/db treated with diazoxide 30mg/kg per day orally from 6 to 18 weeks of age (Dz), iii) db/db treated with pioglitazone 100 mg/kg orally from 6 to 18 weeks of age (Pio) and iv) db/db treated with Dz+Pio, were studied. Body weight and fasted blood glucose (FBG) was measured every two weeks, insulin concentrations (IRI) in plasma and pancreas were also measured, and pancreatic tissue was stained immunohistochemically using antibodies to insulin at 12 and 18 weeks of age.

Results: At 8 weeks of age, FBG in control mice readily showed higher than that in other groups, and further increased in course of time. FBGs in treated groups were lower compared with controls (Dz: 273 ± 60 , Pio: 177 ± 59 , Dz+Pio: 165 ± 53 vs C: 386 ± 103 mg/dl, $p < 0.05$, at 12 weeks of age, and Dz: 284 ± 87 , Pio: 260 ± 30 , Dz+Pio: 225 ± 33 vs C: 722 ± 83 mg/dl, $p < 0.05$, at 18 weeks of age). Plasma IRI levels in treated mice were higher than those in controls (Dz: 5.3 ± 0.9 , Pio: 6.4 ± 1.1 , Dz+Pio: 6.5 ± 1.0 vs C: 5.8 ± 1.2 ng/ml, $p = ns$, at 12 weeks of age, and Dz: 5.5 ± 0.9 , Pio: 6.7 ± 0.8 , Dz+Pio: 7.4 ± 1.1 vs C: 3.3 ± 1.0 ng/ml, $p < 0.05$, at 18 weeks of age). Pancreatic islets and insulin positive cells in control mice were reduced in size and number, while islets were swollen and insulin positive cells were well preserved in mice treated with Dz and/or Pio. This effect on morphological findings was most significant in mice treated with Dz+Pio.

Conclusions: The present results demonstrate that both Dz and Pio prevent the beta-cell damage in db/db mice functionally and histologically, probably through improving insulin over-secretion from beta-cells. Additional effects of Dz and Pio, which mechanisms are different each other, further enlarge the possibility of pharmacological intervention to prevent the progress of diabetes mellitus.

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ALTERED ISLET HORMONE SECRETION IN GRP RECEPTOR DEFICIENT MICE

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Background and aims: Gastrin-releasing peptide (GRP) is a neuropeptide in islet parasympathetic nerves and may therefore be involved in the neural regulation of islet function. This is supported by a previous study where we showed that mice deficient for the GRP receptor gene (GRPR-ko) display impaired glucose tolerance and deficient insulin response after oral glucose. In this study we investigated insulin secretion and insulin sensitivity derived from the minimal model of glucose disappearance after intravenous glucose, as well as glucagon and insulin secretion after autonomic activation in GRPR-ko. **Materials and methods:** The GRPR-ko and controls were given iv glucose (1g/kg), arginine (0.25g/kg), 2-deoxy-glucose (2DG, 0.5g/kg) and carbachol (0.53μmol/kg). Insulin secretion was the suprabasal acute insulin release the first 5 minutes (AIR) and glucose tolerance was the slope of glucose disappearance in 20 min (K_G); insulin dependent glucose disposal was the insulin sensitivity index (S_i). The effect of insulin was defined as the "disposition index" (DI=AIR × S_i). **Results:** The GRPR-ko mice had enhanced insulin response after iv glucose (AIR: 666±72 vs 317±87 pmol/l; p<0.01), and a higher K_G (2.68±0.27% vs 1.81±0.28; p<0.05), but comparable S_i. The DI was enhanced in GRPR-ko mice (7220±1510 vs 2970±740; p<0.05). Furthermore, the GRPR-ko mice displayed deficient glucagon and insulin responses to 2DG (AUC_{glucagon}: 4150±490 vs 7230±750 pmol/l/50min; p<0.01; AUC_{ins}: 35±9 vs 63±14 nmol/l/50min; p<0.05). Glucagon release stimulated by the muscarinic receptor agonist carbachol was the same (AUC_{glucagon}: 590±143 vs 681±205 pmol/l/20min; n.s.) but the insulin response was potentiated in GRPR-ko (AUC_{ins}: 18622±2871 vs 11580±1203 pmol/l/20min; p<0.05). In contrast, the insulin and glucagon responses to arginine were not different between the groups. **Conclusions:** GRPR-ko have an increased glucose elimination after iv glucose due to potentiated insulin secretion with preserved insulin sensitivity. Increased sensitivity to cholinergic stimulus, after a long-standing glucose intolerance, might explain this potentiation. Furthermore, GRPR is essential for islet hormone release after autonomic activation.

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ALTERATION OF BETA-CELL CONSTITUTIVE NO SYNTHASE IS INVOLVED IN THE ABNORMAL INSULIN RESPONSE TO ARGININE IN A NEW RAT MODEL OF TYPE 2 DIABETES.

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Background and Aims: We have previously obtained a new diabetic syndrome in adult rats given streptozotocin and nicotinamide, characterized by reduced beta-cell mass, partially preserved insulin response to glucose and tolbutamide and excessive sensitivity to arginine. In this study, we explored the kinetics of glucose- and arginine-stimulated insulin release in perfused islets as well as the effect of L-N-nitro-L-arginine methyl ester (L-NAME), inhibitor of constitutive NO synthase (cNOS), to provide insight into the possible mechanisms responsible for the arginine hypersensitivity observed in this and other models of type 2 diabetes

Materials and Methods: Perfusion of islets isolated from control and diabetic animals by the collagenase technique.

Results: A reduced first phase and a blunted second phase were observed upon glucose stimulation of diabetic islets, substantially confirming previous data in the perfused pancreas. Exposure of diabetic islets to 10 mM L-arginine, at 2.8 mM glucose, elicited a remarkable monophasic increment in insulin release, which peaked at 639±31 pg/islet/min as compared to 49±18 pg/islet/min in control islets (p<<0.01). Furthermore, the insulin response to arginine was not modified by the addition of L-NAME in diabetic islets, whereas it was markedly enhanced in control islets, as expected in consideration of the documented inhibitory effect exerted by cNOS activity in normal beta-cells.

Conclusions: Our results, obtained through a pharmacological approach, provide for the first time evidence that functional abnormalities of type 2 experimental diabetes, such as the insulin hyper-responsiveness to arginine, could be due to an impairment in cNOS activity of beta-cells.

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Insufficient beta cell proliferation in OLETF (Otsuka Long Evans Tokushima Fatty) rats after partial pancreatectomy: Decreased proliferation or Increased apoptosis?

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Background and Aims: Compensatory beta-cell proliferation for adapting to increased insulin resistance might be achieved by neogenesis of beta-cell from duct cells, replication of pre-existing beta cells and also inhibition of beta-cell apoptosis. This study was designed to study about the mechanism of incomplete beta-cell compensation in OLETF rat after partial pancreatectomy (Px).

Materials and Methods: 12week-old OLETF (Otsuka Long Evans Tokushima Fatty) rats weighing 280-320g were used. 70% partial Px was done. Experimental animals were divided into 4 subgroups by date of killing after Px: 0(Px0), 3(Px3), 90(Px90), 120(Px120) days. After glucose tolerance test (GTT), pancreas remnant was excised and immunohistochemical staining was done for insulin to quantify the beta cell mass by point-counting method and also observed the amount of fibrosis of the islets after Masson's trichrome staining. Immunostaining with anti-BrdU antibody and propidium iodide (PI) of pancreatic islets was analyzed for evaluation of beta-cell proliferation and apoptosis.

Results: We observed that impaired glucose tolerance or diabetes were developed after 70% Px. The mean value of AUC_G of Px0, Px3, Px90 groups in OLETF and LETO rats was 601.9±26.5, 756.6±136.9, 1248.9±219.9mg/dl and 594.5±38.8, 737.3±127.8, 1101.3±206.7mg/dl respectively. In Px0, Px3, Px90 group, the mean value of beta-cell mass was 1.10±0.3, 0.61±0.3, 1.35±0.8mg in OLETF rats and 1.13±0.3, 0.49±0.2, 1.94±0.8mg in LETO rats, but was not statistically different. At Px3, the mean areas of duct cell proliferation were not significantly different between OLETF and LETO rats. And rapidly proliferating duct cells were observed in the adjacent area of common pancreatic duct and main duct even upto 90days after Px. But the amount of fibrosis area in the islets were significantly increased in OLETF rats. In addition, increased PI proportion of beta cells in islets was also observed.

Conclusions: More severe hyperglycemia and islet disorganization were apparent in OLETF rats despite of existence of beta cell regeneration and renewal process. So it seemed that hyperglycemia accelerated aging process or senescence of beta cells in OLETF rats. Increased apoptosis might be an important mechanism for progression of hyperglycemia.

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A STUDY ON THE EFFECT OF NEUROPEPTIDE Y ON PANCREATIC ISLET CELL FUNCTION IN NORMAL AND DIABETIC RATS AND CHINESE HAMSTERS

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Background and Aims: The relationship of neuropeptide Y (NPY) and diabetes is unclear. Thus, we conducted this study to explore the implications of NPY in the regulation of islet cell function in normal and diabetic status. **Materials and Methods:** Pancreatic islets from normal, STZ diabetic rats and diabetic Chinese hamsters were isolated, purified and cultured. Islets were subsequently subjected to stimulation of 11.1mM glucose, and NPY in concentrations ranging from 0.1nM to 10nM for normal islets, and 100nM for diabetic islets by using a column islet cell perfusion system. Insulin and glucagon release in response to glucose and NPY stimuli were evaluated sequentially and dynamically from each collection of perfusate. Besides, islets from diabetic hamster incubated with anti-NPY antibody, or normal rabbit anti-serum for 2h were also perfused and hormonal release assessed in the same ways. **Results:** 1. Low dose (0.1nM) NPY stimulated (79.21±6.52 vs. 121.00±18.20 mU/50 islets, P<0.05), but high dose (>6nM) inhibited (79.21±6.52 vs. 14.12±2.23 mU/50 islets, P<0.01) glucose-stimulated insulin secretion, showing the dual regulatory effects of NPY on insulin release in a dose-dependent manner in both normal rats and Chinese hamsters. 100nM of NPY remarkably inhibited insulin secretion from islet cells in both normal rats and hamsters (131.47±24.60 vs. 68.89±10.70, and 92.88±26.04 vs. 61.81±8.18 mU/50 islets, respectively, P<0.05) and their diabetic models (31.89±10.34 vs. 14.72±8.89, and 89.33±12.59 vs. 53.91±9.18 mU/50 islets, respectively, P<0.05). 2. Insulin release in response to 2.8mM and 11.1mM glucose in anti-NPY antibody pretreated islets significantly increased by 78% and 103%, respectively, compared with normal rabbit antiserum treated controls in diabetic hamsters. 3. High dose NPY (100nM) moderately inhibited glucagon release in both diabetic models (46.75±10.06 vs. 44.48±10.12 ng/50 islets, and 47.98±7.77 vs. 44.11±9.60 ng/50 islets, respectively). **Conclusions:** Our results suggest that NPY may play an important role in the regulation of islet function, and may be involved in the development of diabetes mellitus (supported by grant of National Natural Science Foundation of China, # 39770351). * Present address: Sichuan Provincial Hospital.

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DECREASED RESIDUAL INSULIN SECRETORY CAPACITY IN A NOVEL LARGE ANIMAL MODEL OF TYPE 2 DIABETES

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Background and Aims: A method for induction of impaired glucose tolerance and type 2 diabetes in the Göttingen minipig has previously been published. In the present study, the insulin secretory capacity to glucose and arginine stimuli in this model was compared to that of normal and type 1 diabetic animals.

Materials and Methods: Male minipigs, 11 months, 16-22 kg, were instrumented with permanent jugular vein catheters. To induce diabetes, animals were dosed i.v. with either nicotinamide (NA) 67mg/kg plus streptozotocin (STZ) 125mg/kg (n=11) or STZ (125mg/kg) alone (n=3). Fourteen animals served as normal controls. At least 3 weeks after induction of diabetes a test of insulin secretory capacity was performed: Glucose injections of 300 mg/kg and 600 mg/kg were given at baseline plasma glucose (PG) and an arginine injection (67mg/kg) was given at high PG levels (approx 20-25 mM), all injections were administered intravenously. PG was measured during the whole test and glucose disappearance rates calculated.

Results: Acute 10 min insulin responses (AIR) to glucose and arginine injections are expressed as area under the curve (AUC). Data are presented as means \pm SEM. Insulin secretory capacity in response to 300 mg/kg glucose was decreased significantly in both NA+STZ (1178 \pm 197 pmol/l*min, p<0.001) and STZ (260 \pm 127 pmol/l*min, p<0.001) compared to normal (3251 \pm 215 pmol/l*min). Similarly, the response to 600 mg/kg glucose was decreased significantly in both groups: NA +STZ (1467 \pm 311 pmol/l*min, p<0.001) and STZ (276 \pm 164 pmol/l*min, p<0.001) compared to normal (3593 \pm 335 pmol/l*min). The response to arginine 67 mg/kg was also significantly impaired in the NA+STZ group (1460 \pm 303 pmol/l*min, p<0.01) and in the STZ group (705 \pm 618 pmol/l*min, p<0.05) vs. normal animals (3283 \pm 496 pmol/l*min). However, the values obtained for diabetic animals in the NA+STZ group were consistently higher than those of the diabetic animals in the STZ group. The glucose disappearance rate $K_{\text{sub-G}}$ as measured during 1-10 min after the first glucose bolus of 300 mg/kg was significantly decreased in NA+STZ (3.9 \pm 0.5 %/min, p<0.05) and in STZ (2.5 \pm 0.9 %/min, p<0.01) as compared to normal animals (5.4 \pm 0.2 %/min).

Conclusions: The NA+STZ minipig, with decreased residual insulin secretory capacity and impaired glucose tolerance, holds promise as a new large animal model of type 2 diabetes and could be very useful in the development of new insulin secretagogues or sensitizers for the treatment of type 2 diabetes.

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Pancreatic ductular b-cells and extra-islet b-cell clusters are increased in obese but not in diabetic Macaca mulatta monkeys.

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Background and Aims: Decreased b-cell mass or reduced neogenesis has been proposed as causal factors for reduced insulin secretion in rodents models of Type 2 diabetes. Morphological data from human post-mortem pancreas suggest that b-cell mass is relatively unchanged in Type 2 diabetic subjects but that b-cell neogenesis is increased. To determine the relationship of changes in the b-cell population to the pathophysiology of diabetes, post-mortem pancreas from spontaneously diabetic Macaca mulatta monkeys was examined.

Materials and Methods: Pancreatic specimens taken at post-mortem from 18 monkeys (aged 10-18y) at different stages of the diabetic syndrome were examined by quantitative immunohistochemistry. Animals were assigned to 3 groups defined by their pathophysiology; Group I: non-diabetic lean (n=4), Group II: obese hyperinsulinemic, normoglycaemic (n=7), Group III: Diabetic (D)(n=7). Histological sections were labelled for CK19 (ductal cells) and insulin. Morphometry included counting and area density measurement (IBAS system) of ductal b-cells, extra-islet b-cell clusters (<10 cells) and islet areas which were expressed in relation to tissue sectional area.

Results: Ductal b-cells and extra-islet b-cell clusters were present in all groups and islet amyloid was present in Group III. There was no change in total islet mass/pancreas in Group III, compared to Group I, but there was a significant increase in b-cell mass in group II (obese); both the ductal b-cell number and area density of extra-islet b-cell clusters was significantly increased in Group II compared to Groups I and III (p<0.05). Extra-islet b-cell clusters correlated with fasting insulin (r=0.57; p<0.05).

Conclusions: Obesity and insulin resistance are associated with increased pancreatic b-cell proportion. It is unclear if this increase results from hypertrophy or neogenesis and if the extra-islet b-cells are functional and contribute to increased insulin secretion

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Impairment of induced T cell proliferation in patients with type-1 diabetes

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Background and Aims: Patients suffering from type-1 diabetes have been assigned a higher risk for certain infectious diseases although clinical data still remain controversial. The aim of our study was to investigate a possible impairment of the adaptive immune responses in vitro in patients with type-1 diabetes.

Materials and Methods: A group of patients with type-1 diabetes (n= 34; age: 31,7 \pm 8,8 yrs; HbA1c: 7,2 \pm 0,9%; duration of diabetes: 14,6 \pm 9,8 yrs) were compared to a group of age matched healthy controls (n= 38). Monocyte derived dendritic cells (MDDC) were used as antigen presenting cells (APC).

Results: A significant reduction in the proliferative response of CD4+ T cells to the primary protein antigens keyhole limpet hemocyanin (KLH) and sperm whale myoglobin (SWM) could be observed in patients with type-1 diabetes compared to controls (p<0,05). The stability of MHC class-II-peptide complexes measured by resistance to denaturation of the alpha, beta-dimer in SDS, was significantly reduced for HLA-DR3 and/or DR4-peptide complexes in patients with type-1 diabetes. After stimulation of mononuclear phagocytes of patients with type-1 diabetes with lipopolysaccharide (LPS) significantly elevated amounts of the inhibitory cytokine (IL)-10 were produced (p<0,01) while levels of IL-12 and the inflammatory cytokines tumor necrosis factor (TNF)-alpha and IL-1beta were comparable in both groups.

Conclusions: Taken together these data prove that an impairment of antigen induced T cell proliferation is present in patients with type-1 diabetes. The reduced stability of the MHC class-II-peptide complex as well as an increased expression of the inhibitory cytokine IL-10 may help to clarify the underlying mechanisms of this finding.

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CLINICAL REMISSION IN TYPE 1 DIABETES: ASSOCIATION WITH CHANGES IN CD4+ T CELLS SUBSETS AND NOT WITH ISLET CELL ANTIBODY AND GLUTAMIC ACID DECARBOXYLASE ANTIBODY LEVELS

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Background and Aims: It was previously suggested that appearance of clinical remission (CR) in recent-onset Type 1 diabetes depended on the changes in CD4+ T lymphocyte subset ratio and in beta cell-specific autoantibody levels. The aim of this one year follow-up study in 61 recent-onset Type 1 diabetes patients was to compare changes in CD4+ T cell subsets, islet cell antibody (ICA) and glutamic acid decarboxylase antibody (GADA) levels in peripheral blood between two groups of these patients (pts), group A: pts exhibiting CR (N=32) and group B: pts not exhibiting CR (N=29) during the study period.

Materials and Methods: In group A, the follow-up analysis was done (a) in initial insulin-requiring state (IRS/A I); (b) at day 30 of CR; (c) in relapse of IRS after CR (IRS/A II) and (d) at day 365 (IRS/A III). CR was defined as euglycemia without insulin lasting >30 days (CR duration, 127 \pm 53 days). In group B, the follow-up analysis was done (a) in IRS/B I and (b) at day 365 (IRS/B II). The percentage of memory (CD45RO+) and naive (CD45RA+) CD4+ T cell subsets was analyzed by two-color immunofluorescence staining and flowcytometry. ICA levels were determined by indirect immunofluorescence and GADA levels by ELISA.

Results: We found that in CR percentage of CD4+CD45RO+ T cells was lower compared to IRS/A I, IRS/A II and IRS/A III in group A (29.4 \pm 2.1 vs 35.3 \pm 2.3%, 34.3 \pm 2.3% and 35.7 \pm 2.1%, respectively, p<0.05) as well as to IRS/B I and IRS/B II in group B (33.8 \pm 2.2% and 32.8 \pm 2.8%, respectively, p<0.05). Moreover, in CR, percentage of CD4+CD45RA+ T cells was higher compared to IRS/A I, IRS/A II and IRS/A III in group A (25.2 \pm 1.6 vs 22.6 \pm 1.4%, 22.8 \pm 1.6% and 23.0 \pm 1.5%, respectively, p<0.05) as well as to IRS/B I and IRS/B II in group B (21.9 \pm 1.6% and 22.7 \pm 1.8%, respectively, p<0.05). The ICA and the GADA levels did not differ between CR and neither of insulin-requiring states during the whole study period.

Conclusions: The results of our follow-up study have shown that appearance of CR was strongly dependent on decrease in CD45RO+ (memory cell) and increase in CD45RA+ (naive cell) subset of CD4+ T lymphocytes and it was not related neither to the changes in ICA nor GADA levels.

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NK-CELLS AND MACROPHAGES INFLUENCE T-HELPER LYMPHOCYTE MEDIATED β -CELL DESTRUCTION IN BB RATS

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Background and Aims: Previous studies have shown that besides autoreactive T_H lymphocytes also NK-cells and macrophages have the potential to destroy pancreatic β -cells. However, the relevance of NK-cells and macrophages for the development of type-1 diabetes is still unknown. Therefore, we investigated the appearance of these β -cell reactive cell subsets in dependence on the age of normoglycaemic (nBB) compared to newly diagnosed BB rats (dBB). **Materials and Methods:** Splenic T_H and NK-cells were enriched by magnetic beads loaded with the monoclonal antibodies W3/25 and 10/78. Macrophages were prepared by peritoneal lavage. The cytolytic reactivity was measured with the ^{51}Cr -release assay using prelabelled pancreatic islets as targets. Reactivities of lymphoid cells were calculated β -cell reactive if the ^{51}Cr -release was higher than the upper limit of normal range (mean+2SD of cells from dLEW.1W rats). **Results:** In 35 days old nBB rats neither autoreactive T_H nor β -cell reactive NK-cells or macrophages were observed. At an age of 75 days, the incidence of rats with autoreactive T_H cells was 46%. It increased to 57%, 63% and 81% in 85, 95 and 115 days old nBB and to 88% in dBB rats. β -cell reactive NK-cells were detectable preferentially in dBB, 95 and 115 days old nBB rats (50%, 25%, 45%), however, only in combination with autoreactive T_H cells. Peritoneal macrophages with β -cell reactivity were observed in 33% of 95 and 50% of 115 days old nBB rats. However, not in all these rats an additional T_H cell autoreactivity was found. **Conclusion:** We conclude that because of the discrepancy between the diabetes incidence of 35% and the presence of autoreactive T_H lymphocytes in nearly all diabetes-susceptible rats, autoreactive T_H lymphocytes are necessary but not sufficient for the diabetes manifestation. Additional factors possibly the activation of other cells especially NK-cells may contribute to the diabetes manifestation by the release of soluble mediators destroying β -cells or influencing the reactivity of autoreactive T_H cells.

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IMMUNOLOGIC PHENOTYPE OF LYMPHOCYTES IN OBESE AND LEAN SUBJECTS WITH AND WITHOUT DIABETES MELLITUS

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Background and Aims: Recently immunological abnormalities have been implicated in the pathogenesis of type 2 diabetes mellitus (NIDDM) and/or metabolic syndrome. However, the immunological phenotype of lymphocytes (IPL) in obese and non-obese diabetic and non-diabetic subjects remains not investigated. Therefore, the aim of our study was to investigate IPL in obese and lean subjects with and without NIDDM.

Materials and Methods: We examined 32 obese subjects - 17 with NIDDM (age - 53.9 \pm 2.2 years; BMI - 31.8 \pm 0.7 kg/m²; mean \pm SEM) and 15 without NIDDM (age - 52.3 \pm 1.8; BMI -32.9 \pm 1.1kg/m²) and 25 non-obese subjects - 13 with NIDDM (age - 48.5 \pm 2.1; BMI -24.5 \pm 0.6kg/m²) and 12 without diabetes (age - 49.3 \pm 2.1; BMI - 24.0 \pm 0.4kg/m²). IPL (CD3+, CD4+, CD8+, CD20+, CD56+ cells) was analyzed by fluorescence-activated flow cytometry (FACS tar plus, Becton Dickinson, USA). The number of large granular lymphocytes (LGL) was determined in the blood samples painted by Pappenheim (pH - 6.85) calculating on 200 cells. Statistical analysis was performed by Student's paired test.

Results: We found that the number of CD3+, CD4+, CD8+ cells were significantly higher in obese diabetic patients compared to either diabetic non-obese or obese non-diabetic subjects. However, there was no difference in number of these cells between obese and lean non-diabetic people. The number of CD20+, CD56+ cells did not differ significantly between groups studied. The absolute number of LGL was the highest in obese diabetic patients - 0.40 \pm 0.06 \times 10⁹/L vs. 0.24 \pm 0.04 \times 10⁹/L in patients with diabetes with and without obesity, and 0.17 \pm 0.03 \times 10⁹/L vs. 0.28 \pm 0.04 \times 10⁹/L in those non-diabetic ones with and without obesity, respectively (p<0.05 between obese diabetic subjects and non-obese diabetics and obese non-diabetics).

Conclusions: NIDDM along with obesity could be associated with some immunological abnormalities reflected by changes of IPL. The etiological significance of these changes for the development of NIDDM in obese subjects remains to be established by further studies.

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Analysis of Structure and Function of the binding of an autoantigenic peptide of insulin to CD8 T cells in diabetes.

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Background and Aims: CD8 T cells play an important role in the early phases of pathogenesis of type 1 diabetes. We have isolated a highly pathogenic CD8 T cell clone from the islets of young Non Obese Diabetic (NOD) mice found to recognise insulin B chain amino acids 15-23 (LYLVCGERG) restricted by the MHC class I molecule Kd. The recognition of MHC peptide complexes by T cells is governed by structural considerations that are determined by the sequences of the individual components and their interaction with each other. We have studied peptides that are altered at different positions in the peptide sequence and related this to modelled structures of the MHC peptide TCR complex. Homology modelling is a tried and tested method of approximating the 3-dimensional structure of proteins.

Results: The insulin peptide seems to fit well into the MHC groove with the position 2 Tyr providing the primary anchor, and the position 9 Gly the secondary anchor, mainly through its free carboxylate end. Binding of the native peptide to the MHC is poor and we have shown that substitution of the peptide at positions 5, 7 and 9 is possible due to interactions that stabilise peptide binding, but that alterations at other positions along the peptide are poorly tolerated, related to the fact that positions 1, 3, 4, 5, 6 and 8 appear to be TCR contact residues. Our models demonstrate why the native peptide does not bind well to the MHC and indicate the reasons why it is not possible to substitute particular amino acids in the sequence.

Conclusions: Our current studies have shown that the predictions seen in the models correlate closely with the observed effects in functional assays and enables an understanding of how the MHC, peptide, and TCR interact to be seen from a structural point of view and to be related to the function of these molecules.

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Can the Patients with the Autoimmune Hepatopathy Develop Type 1 Diabetes?

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Background and Aims: Last year we detected an unexpected high number of the patients with the autoimmune hepatopathy (AH), who had positive markers of the autoimmune insulinitis. The aim of our next work was to detect an impaired first phase insulin secretion in patients with AH and to compare the results of the patients with positive and negative markers of the insulinitis.

Materials and Methods: We examined 38 patients with the primary biliary cirrhosis (PBC) or the autoimmune hepatitis (AIH). We proved positive markers of the insulinitis in 22 (58%) persons. The detected markers were ICA-islet cells antibodies, IAA-insulin autoantibodies, GADA-glutamic acid decarboxylase antibodies and IA-2A-tyrosinphosphatase antibodies. We performed an intravenous glucagon test (IVGT) to all 38 patients. 1 mg of glucagon (NOVO Nordisk) was administered intravenously and C peptide levels were tested before and 6 minutes after the administration. The low rise of C peptide (below 1,2 nmol/l) was an indicator of the impaired first phase insulin secretion.

Results: 24 (63%) patients had a normal IVGT. 11 persons from this subgroup had positive markers of the insulinitis and 13 persons had no marker of the insulinitis. 14 (37%) patients had an impaired IVGT. 10 persons from this subgroup had positive markers of the insulinitis and 4 persons had no marker of the insulinitis (p=0,08). The patients with GADA positivity had an impaired IVGT in 6 cases from 9 (p=0,05). There was no significant difference in IVGT in patients with ICA, IAA and IA-2A positivity or negativity.

Conclusions: We proved a significant impaired first phase insulin secretion in patients with GADA positivity. We did not prove this in patients with other markers of the insulinitis. Nobody of our patients have developed diabetes yet.

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Effect of acutely induced hyperinsulinemia and hyperglycemia on phagocytosis and oxidative burst of polymorphonuclear cells in healthy subjects.

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Background and Aims: High medium concentration of glucose impairs some functions of polymorphonuclear cells (PMN) in studies realized in vitro. The effect of in vitro induced hyperinsulinemia on immune functions has not been well documented yet. The aim of our study was to assess the effect of acutely induced hyperinsulinemia and/or hyperglycemia on phagocytosis and oxidative burst of PMN in vivo.

Materials and Methods: Our study was performed on 10 healthy subjects (mean age 27±6 years, mean BMI 24±3 kg/m²). Healthy subjects underwent four different 4 hours lasting clamp studies. Acute hyperinsulinemia and/or hyperglycemia (17 mmol/l) were induced by 1. hyperinsulinemic (60 mU/l) euglycemic clamp (HEC), 2. by hyperglycemic hyperinsulinemic clamp (HHC), 3. by hyperglycemic clamp with insulin secretion blockade (HC) following somatostatin application. As a control clamp study (4.), we used time and volume controlled saline infusion. PMN phagocytic activity (mean percentage of phagocytizing PMN, mean fluorescent intensity of phagocytizing PMN) and oxidative burst (mean percentage of active PMN, mean fluorescent intensity of active PMN) were measured before and after clamp studies by flow cytometry under basal condition and after *Escherichia Coli* stimulation (PHAGOTEST, BURSTTEST, Orpogen Pharma, Heidelberg, Germany).

Results: No significant differences in PMN functions as parameters of PMN phagocytosis (mean percentage of phagocytizing PMN 5.1±4% vs 5.6±5.2% (NS) and mean fluorescent intensity of phagocytizing PMN 171±457 vs 282±573 (without unit) (NS) under basal condition, before vs after clamp study, respectively) and parameters of PMN oxidative burst (mean percentage of active PMN 3.7±2.5% vs 2.7±2.3% (NS) under basal condition and 93.6±12.3% vs 86.5±28.4% (NS) after *E.Coli* stimulation; mean fluorescent intensity of active PMN 329±166 vs 433±350 (NS) under basal condition and 2362±1282 vs 1960±1321 (NS) after *E.Coli* stimulation, before vs after clamp study, respectively) were found during HEC. Similarly, values of these parameters before and after the HHC and HC clamp studies did not differ significantly and were not different from control values found during controlled saline infusion as well.

Conclusions: The nonspecific immune functions of PMN (phagocytosis and oxidative burst) were not influenced by short-time isolated hyperinsulinemia, by simultaneous hyperglycemia and hyperinsulinemia or by isolated hyperglycemia during in vivo clamp studies in healthy subjects. Supported by IGA grant No. NB/5323-3 of the Czech Ministry of Health

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GALACTOSYL CERAMIDE (GAL-CER) INCREASES AND SULFATIDE DECREASES CYTOKINE PRODUCTION OF WHOLE BLOOD CELLS.

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Aim: To test the effect of sulfatide and its precursor gal-cer (galactosylceramide) on cytokine production of whole blood culture from healthy donors. In β -cells sulfatide and gal-cer are present in similar amounts, but sulfatide is known to be secreted together with insulin.

Methods: PHA or LPS stimulated diluted whole blood cells were incubated with 30 μ mol/l of sulfatide or gal-cer. Furthermore, competition assays with both sulfatide and gal-cer in various concentrations were performed. After 24 hrs supernatants from the cultures were harvested and the levels of IL-1 β , IL-6, Interferon- γ (INF- γ), and TNF- α were determined using ELISA.

Results: Sulfatide decreased production of the investigated cytokines (all data in percentage of control levels): IL-6: 42 %, and INF- γ : 65 %. In contrast, gal-cer stimulated cytokine production as compared to control levels: IL-1 β : 371 %, IL-6: 312 %, INF- γ : 489 %, and TNF- β : 1528 %.

When sulfatide and gal-cer were present simultaneously in concentrations of 30 μ mol/l the cytokine productions were: IL-1 β : 340 %, IL-6: 272 %, INF- γ : 288 %, and TNF- β : 1040 %. Sulfatide in concentrations of 120 μ M reduced the effect of 30 μ mol/l gal-cer to: IL-1 β : 278 %, IL-6: 222 %, INF- γ : 194 %, and TNF- α : 1010 % of control levels.

Conclusion: We have demonstrated that gal-cer increases and that sulfatide decreases the production of cytokines known to be toxic to β -cells. Furthermore, gal-cer seems to overrule the effect of sulfatide. We speculate that leakage of gal-cer in the islet environment might be potentially harmful to the β -cells.

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CYTOKINE PROFILES AND IgG-IA SUBCLASSES RESPONSE IN TYPE 1 DIABETIC PATIENTS TREATED WITH ORAL INSULIN.

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Background and Aims. Tolerance to oral administered antigens may be generated through the induction of Th2/Th3 regulatory cells. We recently reported that oral insulin administration (5 mg daily for one year) starting at the time of diagnosis in Type 1 diabetic patients had no effect on residual beta cell function. The aims of this study was to evaluate whether the above treatment determined the induction of regulatory T cells as measured by cytokine profiles and a Th2-like antibody response.

Materials and Methods. IFN γ , IL-4, IL-5 and TGF β cytokines were measured in insulin and PHA stimulated lymphocytes supernatants by ELISA every 3 months.

IgG subclasses of insulin antibody were measured by RIA. **Results.** TGF β production was statistically higher in patients treated with oral insulin ($n=11$) compared with patients receiving placebo ($n=9$) at 12 months from diagnosis ($p=0.01$ and $p=0.004$ for lymphocytes challenged with insulin or PHA, respectively). Levels of IL-4, IL-5 and IFN- γ were similar between the two treated patient's groups both at baseline and after stimulus with the antigens. IgG1 and IgG3 insulin antibody subclasses were statistically lower in patients treated with oral insulin compared to placebo control group after 12 months ($p=0.02$ and $p=0.01$, respectively).

Conclusions. The higher level of TGF β in patients treated with oral insulin showed that immunoregulatory T cells are induced by this treatment. The lower IgG1 and IgG3 antibody response in oral insulin treated-patients is consistent with a Th2 deviation of the immune response. The failure to achieve any measurable clinical benefit may be due to the timing of treatment implementation.

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IMMUNE RESPONSES AGAINST RAT CYTOMEGALOVIRUS IN DIABETES PRONE AND DIABETES RESISTANT BB RATS

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Background and Aims: Recently, we showed that infection with RatCytomegalovirus (RCMV) of Diabetes Prone (DP) BB rats resulted in significantly accelerated development of diabetes. The mechanism by which RCMV accelerates the development of diabetes is still unknown, however cytolytic infection of beta-cells is unlikely. Stimulatory effects of RCMV on the T cell repertoire of DP BB rats might influence the diabetogenic process. To further characterize the immune response against RCMV, in vitro as well as in vivo experiments were performed after RCMV infection of DR and DP BB rats.

Materials and Methods: The in vivo immune response against RCMV was studied in immunocompetent DR BB rats. Rats were left untreated or i.p. infected with 1x10⁶ pfu RCMV (Maastricht strain). Animals were sacrificed 6, 12 and 18 days p.i. and flowcytometry was performed on splenocytes and PBMC's using the following mAb's: R73 (TCR), OX35 (CD4) and OX8 (CD8). To further analyze T cell responses in BB rats after RCMV infection, in vitro proliferative responses of DR and DP splenocytes were measured 21 days p.i. and compared to the responses observed after ConA stimulation. Finally, the presence of anti-RCMV antibodies in serum of DP and DR BB rats after RCMV infection was determined.

Results: Although not significant, RCMV infection resulted in an increase in the percentage CD8+ T cells 12 and 18 days p.i. Three weeks p.i., DR splenocytes showed RCMV specific proliferation after stimulation with fixed autologous infected fibroblasts (68±176 dps), whereas stimulation with uninfected fibroblasts did not result in T cell proliferation (49±7 dps, background levels). DP BB derived splenocytes did not show RCMV specific T cell proliferation. The magnitude of RCMV specific T cell proliferation in DR BB rats was about 40-50% of the proliferation observed after polyclonal ConA stimulation. These results indicate that a high proportion of the T lymphocytes is responsive to stimulation with RCMV in vitro, suggesting polyclonal activation of T cells by RCMV. Infection with RCMV resulted in generation of significant titres of RCMV specific antibodies in both DP and DR BB rats.

Conclusions: RCMV infection of DR BB rats resulted in the generation of strong proliferative T cells responses in vitro, suggesting polyclonal T cell stimulation. Although not detectable in DP BB rats in vitro, such activation of (autoreactive) T cell might be involved in the accelerated development of diabetes in DP BB rats after RCMV infection.

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Smokers show a higher degree of immune activation at each level of adiposity.

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Background and Aims: The independent contribution of smoking, as a confounding factor of the relationship between fat mass and immune activation has not been evaluated, despite the evidences that smoking alters cytokine production. As obesity 'per se' is recognized as a chronic inflammatory state, we speculated a higher degree of immune activation in smokers at each level of adiposity.

Patients and methods: We evaluated the serum concentration of soluble tumor necrosis factor- α receptors (sTNFR1 and sTNFR2), insulin and leptin in a population of 264 healthy subjects.

Results: We found a steeper relationship between circulating sTNFR1, sTNFR2 and body fatness among smokers. Smoking men were similar in age, BMI and WHR, and had significantly lower fat mass, lower fasting glucose, insulin and leptin concentrations than non-smoking men. Of note was that smoking men also showed significantly higher circulating sTNFR2 levels than non-smoking men (3.7 ± 0.8 vs 3.4 ± 0.7 ng/mL, $p=0.03$). A tendency towards lower fasting glucose and higher sTNFR2 concentration were also observed in smoking compared with non-smoking women. The relationship between BMI and sTNFR1 was not statistically significant among non-smokers.

Conclusion: Smokers show a higher degree of immune activation, as defined by higher serum sTNFR2 concentration, at each level of adiposity. The findings of the present study may help to understand the apparent paradox of increasing TNF- α activity of obesity.

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THE PREDICTIVE VALUE OF THYROID-ANTIBODIES AND TSH-LEVELS IN A LONGITUDINAL STUDY OF FIRST-DEGREE RELATIVES OF TYPE 1 DIABETIC PATIENTS

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Background and Aims: Thyroid autoimmunity is often associated with type 1 diabetes mellitus. We studied the prevalence of thyroid-Abs (TG/TPO) as well as the TSH levels basal and after 7yrs in first-degree relatives of type 1 diabetic patients, in order to evaluate the predictive value of these markers for impaired thyroid function in these subjects. **Materials and Methods:** We determined islet-cell autoantibodies (ICA, IA-2ic, GAD 65), thyroid-Abs and TSH levels basal and after 7 years in 425 first-degree relatives of type 1 diabetics (mean age 29.2 ± 14.4 years). The distribution of the relatives was: siblings/children 199 (group A) and parents 226 (group B). **Results:** Thyroid-Abs were found positive in 50 cases overall, 24 (12.06%) in group A and 26 (11.5%) in group B. TSH basal levels were at the upper limit of the normal range (2.5 ± 1.8) for subjects with thyroid-Abs positivity in comparison to those tested negative for TPO. In addition TSH levels after 7yrs were increased to 3.6 ± 1.9 . In group A, a strong positive correlation ($r=0.45$, $p<0.001$) was observed between TPO-Abs and TSH basal levels, i.e. higher TSH levels were observed in subjects with TPO-Abs positivity in comparison to those tested negative for TPO. The above correlation became stronger after 7 yrs ($r=0.53$, $p<0.001$). In the same group, a significant positive correlation was seen between IA-2ic and TPO-Abs positivity ($r=0.31$, $p<0.001$), which also became stronger ($r=0.42$, $p<0.001$) after 7 years. No significant correlation was observed regarding the group B. **In conclusion:** Thyroid-autoimmunity has to be tested regularly and on a long-term basis not only in diabetic patients but also in their first-degree relatives who are at risk for type 1 diabetes mellitus.

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ASSOCIATION OF AUTOIMMUNE MARKERS OF INSULITIS, THYROID AND COELIAC DISEASES IN TYPE 1 DIABETES MELLITUS

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Background and Aims: Type 1 diabetes mellitus as an autoimmune disease may be accompanied by the presence of GAD or IA-2 antibodies. In addition, its association with other autoimmune disorders has been repeatedly observed. The aim of this study was to evaluate the presence of autoantibodies as markers of insulinitis, thyroopathy and coeliac disease in Type 1 diabetic patients. **Patients and Methods:** Fifty Type 1 diabetic patients (20 men/30 women, age 37 ± 11 yrs, diabetes duration 17 ± 13 yrs, BMI 24.4 ± 2.6 kg.m⁻²) were examined in present study. Autoantibodies (Ab) against GAD65 and IA-2 as markers of insulinitis, anti TPO and anti TG as markers of autoimmune thyroid disorder and antibodies against gliadin (IgA, IgG), tissue transglutaminase (ATTG) and endomysium (EMA) as markers of coeliac disease were determined in all patients. RIA kits (Solupharm and Immunotech, Czech Republic) were used for the estimation of antibody concentrations. The results higher than 1 U.mL⁻¹ in anti-GAD65 and anti-IA-2, 50 U.mL⁻¹ in anti-TPO and 100 U.mL⁻¹ in anti-TG, 10 U.mL⁻¹ in ATTG and index higher than 30 in both IgA and IgG anti-gliadin antibodies were considered positive. **Results:** The presence of positive antibody results (Ab+) in separate autoimmunopathies (AIP) as well as the presence of at least one or more combined AIP are shown in the table. In three patients (6%) no positive antibodies were found. No clinical evidence of thyroopathy was observed in

Autoimmunopathy (AIP)	Number of Ab+ AIP			Total n (%)
	1	2	3	
Type 1 DM	8	16	8	32 (64)
Thyroopathy	1	3	8	12 (24)
Coeliac disease	11	19	8	38 (76)
Pts with respective number of AIP	20 (40%)	19 (38%)	8 (16%)	-

10 of 12 antibody positive patients before the biochemical examination. The same was true in 36 of 38 antibody positive patients screened for coeliac disease. In two patients (1 m, BMI 18.6 kg.m⁻² / 1 w, BMI 17.4 kg.m⁻²) all four markers of coeliac disease were positive and subclinical form of coeliac disease was diagnosed.

Conclusion: Antibody positivity against thyroid gland or intestine antigens is very frequent in Type 1 diabetic patients without any evidence of clinical manifestation. The regular screening for different antigens in these patients is therefore recommended to disclose subclinical autoimmunopathy.

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HIGH PREVALENCE OF CELIAC AND AUTOIMMUNE THYROID DISEASES IN SUBJECTS WITH TYPE 1 DIABETES MELLITUS.

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Background and Aims: Diagnosis of unrecognized celiac (CD) and autoimmune thyroid (ATD) diseases is potentially important. The prevalence of these diseases in patients with Type 1 diabetes (DM-1) is uncertain in our area. We report the prevalence of CD and ATD among patients with DM-1 in La Palma island (730 Km², 81.000 inhabitants).

Materials and Methods: Anti-endomysial antibodies (Em-ab) were measured in 100 subjects (52 males, 48 females) with DM-1 (according to the WHO 1.985 or ADA 1.997 criteria) attending our clinic. Anti-peroxidase (TPO) and anti-thyroglobulin (Tg) antibodies were measured as markers for thyroid autoimmunity. All those subjects who tested positive for EM-ab underwent intestinal biopsy. Islet cell (ICA), tyrosine phosphatase (IA2) and glutamic acid decarboxylase (GAD) antibodies were measured as markers for islet cell autoimmunity. To compare variables the Student's t, chi square or Fisher's exact tests were employed.

Results: The mean age of subjects was 27.8 years (range: 5-60 yr) and the mean duration of diabetes was 10.2 yr (range: 0-45 yr). We found Em-ab in 6% of subjects (4 male and 2 female). Only one patient had recognized CD prior study. No differences were observed according to sex. There were no found significant differences between subjects with or without ATD and neither in relation to islet cell autoimmunity. Thyroid autoimmunity were detected in 14% of subjects (14 TPO and 2 Tg). The prevalence of ATD was higher in subjects with islet cell autoimmunity (22% vs 6%; p=0.021). No differences were observed according to sex and neither between subjects with or without CD.

Conclusions: 1) Both prevalences (CD and ATD) are higher than in general population, and at least the ATD is more probable when islet cell autoimmunity is present. 2) Because most cases are clinically unrecognized, consideration should be given to serological screening using the IgA anti-endomysial test (as well as study of thyroid function) in all subjects with DM-1.

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PREVALENCE AND CLINICAL APPLICATION OF ICA, IA2 AND GAD ANTIBODIES IN DIABETIC PATIENTS DIAGNOSED BEFORE 40 YEARS OLD.

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Background and Aims: To know the prevalence of islet cell autoimmunity -islet cell (ICA), tyrosine phosphatase (IA2) and glutamic acid decarboxylase (GAD) autoantibodies (ab)- among diabetic patients under 40 years at the time of diagnosis and to check his utility in diabetes (DM) classification.

Materials and Methods: A total of 125 subjects (60 males, 65 females) were screened for islet cell autoimmunity (ICA, IA2 and GAD ab). All of them were younger 40 years at diagnosis of DM, being the mean age at diagnosis 19.8 years (range 0-44 yr). We compared the prevalence according to sex, age at diagnosis groups (0-29 and 30-39 yr) and duration of DM. Also were compared the Body Mass Index (BMI), age at debut and duration of DM between the subjects with or without autoimmunity. To compare variables were employed the Student's t, chi square or Fisher's exact tests.

Results: We found ab in 46.4% of patients (ICA 3.2%; IA2 20% and GAD 36.8%). No differences were observed according to sex (46.7% in male, 46.2% in female) and neither age at debut (51.6% in 0-29 age group; 32.3% in 30-39 age group; p=0.054). The prevalence according to duration of DM (0-1, 2-5, 6-10 and more than 10 yr) was: 61.1%, 61.8%, 52% and 27.1% (ICA 5.6%, 5.9%, 4% and 0%); (IA2 44.4%, 35.3%, 12% and 4.2%) and (GAD 38.9%, 47.1%, 40% and 27.1%). The BMI, age at diagnosis and duration of DM were smaller in subjects with autoimmunity (-22.1 vs 26.1 kg/m²; p<0.0001); (6.8 vs 12.9 yr; p<0.0001) and (17 vs 22.2 yr; p=0.014) respectively.

Conclusions: 1) The presence of islet cell autoimmunity in diabetic patients diagnosed before 40 yr old is related with BMI, age at presentation and duration of disease. The ab have tendency to disappear, being the GAD ab the most prevalent and persistent. 2) At least 1/3 of patients diagnosed between 30-39 yr have an autoimmunity component. In this group, the presence of ab have direct implications for adequate classification and treatment of DM.

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THYROGASTRIC AUTOIMMUNITY IN FIRST-DEGREE RELATIVES OF TYPE 1 DIABETIC PATIENTS.

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Background and Aims: Approximately 25% of type 1 diabetic patients exhibit thyrogastric antibodies (aTPO, PCA) which may be accompanied by dysthyroidism, iron deficiency or pernicious anaemia. Immune, genetic and clinical factors may help to predict autoantibody status. We studied the prevalence of thyrogastric antibodies and (sub)clinical thyrogastric disease in first-degree relatives in relation to age, gender, HLA-DQ type, β -cell antibodies (ICA, IA2A, GADA) and proband thyrogastric antibody status. **Patients and Methods:** Sera from 272 type 1 diabetic patients (M/F: 116/156; mean age: 27 \pm 18 y; duration: 10 \pm 9 y) and 397 first-degree relatives (M/F: 192/205; parents/siblings/offspring: 48/222/127) were screened for IA2A, GADA and aTPO by radiobinding assays and for ICA and PCA by indirect immunofluorescence. In addition, a peripheral haemogram, iron, gastrin, TSH and fT4 levels were determined. **Results:** GADA were present in 68% and 5%, ICA in 36% and 2.5%, IA2A in 45% and 0.5%, aTPO in 21% and 4.5% and PCA in 18% and 11% of diabetic patients and relatives respectively. aTPO-positivity in relatives was associated with age (β =0.22, p=0.0001) and proband aTPO status (β =2.6, p=0.002). The presence of PCA in relatives was determined by age (β =0.04, p=0.026). Moreover, PCA were more frequent in relatives of PCA+ probands than in relatives of PCA- probands (OR=3.0, p=0.01). HLA-DQ type and β -cell antibody status showed no association with thyrogastric antibody status in relatives. In relatives, (sub)clinical dysthyroidism was diagnosed in 3%, iron deficiency anaemia in 12% (PCA+/PCA-: 26%/9%, p=0.009), and pernicious anaemia in 0.5% (PCA+/-: 5%/0%, p=0.012). They had less thyroid dysfunction (p<0.0001) and pernicious anaemia (p=0.018) than diabetic probands. **Conclusions:** Thyrogastric autoimmunity is more frequent in type 1 diabetes than in their first-degree relatives. Thyrogastric antibody status in relatives is determined by age and proband thyrogastric antibody status, but not by HLA-DQ type or β -cell antibody status.

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REGISTRATION OF POSITIVE AUTOANTIBODIES IN YOUNG TYPE 1 DIABETIC PATIENTS DEPENDING ON DURATION OF DIABETES.

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Background and Aims: The aim was to estimate and compare frequency of registration specific for type 1 diabetes mellitus (DM1) autoantibodies (AB): to glutamic acid decarboxylase (AB-GAD), protein tyrosine phosphatase (AB-IA2) and non-specific AB: to thyroid peroxidase (AB-TPO), thyroglobulin (AB-TG) in young patients with different duration DM1. **Materials and Methods:** We measured AB-GAD, AB-IA2, AB-TPO and AB-TG levels by RIA in the sera of 85 patients (39 males, 46 females) with DM1. Mean age was 12.1 \pm 0.35 yrs (9-16), mean DM1 duration 1.65 \pm 0.13 yrs (0.17-3). We estimate frequency of positive AB levels in the study group as a whole and after dividing all patients in 3 subgroups: 1-st - patients with DM1 duration less than 1 yr; 2-nd - with DM1 duration 1-2 yr; 3-d with DM1 duration 2-3 yr. **Results:** In the study group positive AB-GAD were determined in 83.3% of patients, AB-IA2-in 52.4%, AB-GAD and AB-IA2 simultaneously-in 47.6%. The frequency of positive AB-TPO was 21.4%, AB-TG-14.3%. The data about positive AB depending on the duration of DM1 are presented in the table.

№ subgroup (duration of DM1, yr)	Frequency of positive autoantibodies, %			
	AB-GAD	AB-IA2	AB-TPO	AB-TG
1. (0-1)	100	58.3	8.3	25
2. (1-2)	81.2*	50	18.8	6.3
3. (2-3)	53.9** ^	46.2	38.5*	7.7

* - p < 0.05 in comparison with 1 subgroup (0-1 yr); ** - p < 0.01 in comparison with 1 subgroup (0-1 yr); ^ - p < 0.1 in comparison with 2 subgroup (1-2 yr)

Obtained results shown that frequency of positive AB-GAD is significantly reduced with increasing duration of DM1. The same tendency to decreasing of positive AB-IA2 was observed, however statistically not significant. Also the significant growth of registration positive AB-TPO was marked with increasing of DM1 duration.

Conclusions: Our data demonstrate that frequency of specific for DM1 autoantibodies reduce with the increasing of DM1 duration. At the same time the levels of AB-TPO increased with the time of disease. We may suggest that this data is the indirect proof of increasing risk of autoimmune thyroid disorders occurrence in patients with increasing DM1 duration.

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Autoantibodies and C-peptide level in children with newly diagnosed type 1 diabetes

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Background and Aims: To investigate whether the presence of autoantibodies at diagnosis could be applied to predict the intensity of autoimmune beta-cell destruction, concerning C-peptide levels as a marker of islets damage.

Materials and Methods: 103 newly diagnosed type 1 diabetic children at the age of 2.3-18.2 years (median=11.4) were studied. Islet cell antibodies (ICA) at diagnosis were detected by indirect immunofluorescence test performed on human pancreas sections, blood group 0. Antibodies to glutamic acid decarboxylase (GADA) and tyrosine phosphatase antibodies (IA2A) at disease onset were measured by microradioimmunoassay. Fasting C-peptide levels were examined by radioimmunoassay at clinical diagnosis and after 10 days and after 1, 2, 3, 6 and 12 months of disease.

Results: 81.5% of diabetic children were ICA-positive, 66% of children tested positive for GADA and 62.1% for IA2A. Only 4 subjects (3.9%) had no detectable autoantibodies. Plasma C-peptide levels changed during the first year after clinical diagnosis. Median C-peptide levels peaked at 3 months ($p<0.001$) and declined thereafter. One year after diagnosis C-peptide levels decreased in ICA(+) patients ($p<0.05$) but not in ICA(-) patients. The children initially positive for GADA had also decreased serum C-peptide levels at 12 months ($p<0.01$). There was no significant difference between the IA2A-positive and negative subjects in the C-peptide levels at 12 months of disease.

Conclusions: We conclude, therefore, the presence of diabetes-related autoantibodies may be associated with accelerated beta-cell destruction.

Supported by Polish State Committee for Scientific Research grant No 4PO5E 05516

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TITER AND COMBINATION OF ISLET CELL AUTOANTIBODIES DISCRIMINATE TWO TYPES OF LATENT AUTOIMMUNE DIABETES IN ADULTS

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Background and Aims: The aim of this study was to define immunological parameters for the discrimination of patients with distinct metabolic features in adult latent autoimmune diabetes.

Material and Methods: Sera of 312 patients with short-term diabetes (duration <5 years) diagnosed above age 35 years were screened for ICA, GAD- and IA2A-Ab's by workshop validated antibody assays. The antibody status was correlated with age, body mass index, residual beta cell function measured by fasting C-peptide, onset of diabetes related complications and markers of the metabolic syndrome (hypertension and hyperlipidemia).

Results: Fifty one antibody positive patients were identified. These patients had lower fasting C-peptide ($p<0.01$) and less neuropathy ($p<0.005$) and hypertension ($p<0.025$) compared to matched antibody negative patients. However, only patients with two or more antibodies had reduced residual beta cell function compared to antibody negative ($p<0.002$) or single antibody positive (ICA or GAD-Ab's only, $p<0.005$) patients. Patients with multiple antibodies were also leaner ($p<0.005$) and had less frequently diabetes related complications ($p<0.005$) or hypertension ($p<0.005$) compared to single antibody positive or antibody negative patients. IA2 antibody status did not contribute to diagnosis or differentiation of LADA patients.

Conclusions: The combined appearance of ICA and GAD antibodies and high titer of GAD antibodies are a hallmark for patients with insulin deficiency presenting clinical features of type 1 diabetes (LADA-type 1). Single antibody positivity and low titer antibodies are markers for LADA-type 2 associated with the clinical and metabolic phenotype of type 2 diabetes patients.

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CLINICAL REMISSION IN TYPE 1 DIABETES: PREDICTIVE VALUE OF COMBINED ISLET CELL AND GLUTAMATE DECARBOXYLASE ANTIBODY TESTING AT ONSET OF THE DISEASE

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Background and Aims: Our previous data have suggested that islet cell antibody (ICA) testing at onset of the disease might have a predictive value for appearance of clinical remission (CR) in recent-onset Type 1 diabetes. The aim of this study was to analyse the predictive value of a combined two beta-cell specific antibody testing at onset of disease. Therefore, we compared (a) incidence of CR, (b) duration of CR and (c) rapidity of insulin secretion capacity (ISC) decline of until complete insulin dependency among the following groups of patients characterized by initial ICA and glutamate decarboxylase antibody (GADA) levels: group A (ICA+/GADA+, N=25), group B (ICA+/GADA-, N=17), group C (ICA-/GADA+, N=14) and group D (ICA-/GADA-, N=13).

Materials and Methods: The follow-up of the patients was done during the first two years of the overt disease. CR was defined as euglycemia without insulin lasting >30 days. The autoantibody levels were determined at onset of the disease (<6wk after diagnosis). ICA levels were detected by indirect immunofluorescence on human pancreas cryostat sections and GADA levels by ELISA. The rapidity of ISC decline was measured as a time period until the detection of complete insulin dependency (fasting C-peptide (RIA) <0.2nmol/l).

Results: At onset, we did not find significant differences in ICA levels between groups A and B, nor in GADA levels between groups A and C. During the follow-up, we found that the incidence of CR was significantly lower in groups A and B compared to groups C and D (A: 8/25, B: 5/17, C: 11/14, D: 9/13, A,B vs C,D $p<0.05$, A vs B = NS, C vs D = NS). Similarly, the duration of CR was significantly shorter in groups A and B than in groups C and D (CR duration: A: 92+/-38, B: 106+/-32, C: 184+/-44, D: 197+/-42 days, A,B vs C,D $p<0.05$, A vs B = NS, C vs D = NS). In groups A and B, the time period until complete insulin dependency was significantly shorter than in group B (A: 227+/-37, B: 243+/-45, C: 394+/-57 days vs B: 407+/-49 days; A,B vs C,D $p<0.05$, A vs B = NS, C vs D = NS).

Conclusions: Our results have demonstrated that ICA-positivity at onset of the disease was associated with lower incidence and shorter duration of CR and with more rapid decline of ISC, while the presence of GADA was not related to these parameters. The results imply that combined ICA and GADA testing did not improve the predictive value of ICA detection alone at onset of the disease.

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THREE PATIENTS WITH IMMUNE MEDIATED COMPLICATIONS ASSOCIATED WITH SUBCUTANEOUS INSULIN THERAPY

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Background and Aims: Based on three s.c. insulin treated patients (Pat. 1 with type 1 D.m., Pat. 2 with type 2 D.m., Pat. 3 with secondary D. m. due to partial pancreatectomy) we suggest a diagnostic and therapeutic approach to deal with subsequent immune mediated complications [e.g. insulin antibody (ab) formation and/or severe skin reactions] resulting in poor metabolic control in all of the subjects.

Methods: Beside routine clinical/laboratory testing including c-peptide, HbA1c etc. we performed the following immunological evaluation: a. Intradermal skin testing (+/- controls, different insulin preparations, galenic components), b. Quantification of insulin specific IgG and IgE abs in the serum, c. To determine the clinical relevance of these abs we analyzed the time-dependent binding/dissociation curves of the insulin/abs complexes in an ex vivo/in vitro assay. **Results:** In all of the patients we found high levels of insulin specific IgG abs (normal: <0.03mU/ml), binding approx. 60-70% of the insulin with delayed dissociation (up to 6 hours). In addition, Pat. 3 generated insulin specific IgE abs exhibiting a severe/immediate local skin reaction for all insulin prep. used. In Pat. 2 we identified protamine as the challenging antigen for the local skin reactions. Interestingly, Pat 2 and 3 were found to have high plateau levels of fasting/post-prandial c-peptides potentially induced through a relative insulin shortage because of the large amount of antibody-bound/inactivated insulin.

	pos. intrad. skin testing	insulin specific abs IgG (mU/ml) / IgE (RAST)	insulin / abs bind. / diss.	c-pep.(ng/ml) fast. / post-p
Pat 1	-	IgG: 0.37 / IgE: -	69 % / delayed	<0.1 / <0.1
Pat 2	protamine	IgG: 0.27 / IgE: -	65 % / delayed	9.7 / 9.8
Pat 3	all ins. prep.	IgG: 0.43 / IgE: 3	66 % / delayed	4.1 / 4.5

For Pat.1 we suggested CSII with an insulin analog in order to adjust the insulin delivery to steady state conditions. Pat. 2 was set on prandial insulin therapy (analog, no protamine) only and Pat. 3 (proven to have an endogenous insulin production despite partial pancreatect.) was set on max. OAD therapy without insulin. No more skin reactions were noted and metabolic control improved considerably. **Conclusion:** The proposed combined approach allows differential diagnosis and adopted therapy of different forms of immune mediated complications assoc. with s.c. insulin therapy.

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Metabolic Consequences of Oxidative Stress

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INFLUENCE OF HYPERINSULINAEMIA AND HYPERGLYCAEMIA ON OXIDATIVE STRESS

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Background: Long-term hyperinsulinaemia and hyperglycaemia increase oxidative stress by production of reactive oxygen species (ROS). The aim of the study is to compare effects of short - term hyperglycaemia and short - term hyperinsulinaemia on parameters of oxidative stress in an animal model. **Methods:** 40 male Wistar rats (aged 3 months, weight 300-350g) were used in this study. 20 rats were infused simultaneously with insulin and 30% glucose during the hyperinsulinaemic clamp (100 IU/L) in two different glycaemia levels (6 and 12 mmol/L). 10 rats were clamped on glycaemia 6 mmol/L followed by 12 mmol/L, 10 rats were clamped on glycaemia 12 mmol/L followed by 6 mmol/L. 20 rats were used as a control group and they were infused simultaneously with normal saline and 30% glucose as described above. Measured markers of oxidative stress were malondialdehyde (MDA), glutathione (GSH) and total antioxidant capacity (AOC). The M-value (numeric expression of peripheral tissue insulin resistance) was calculated for quantification of glucose oxidation during the clamp. The results were correlated to total plasmatic protein and compared with the control group. Statistical significance was determined by one way analysis of variance (ANOVA). **Results:** 1. Total antioxidant capacity increased significantly ($p<0.05$) according to the duration of the clamp and independently on the sequence of hyperglycaemic clamp (15,13±1,44 vs 16,84±2,27 and 15,80±0,45 vs 17,83±0,24) and euglycaemic clamp (14,31±1,10 vs 15,92±2,10 and 15,20±0,50 vs 18,48±0,38). There is a statistically significant difference in comparison with the control group (the second two values in the brackets). 2. Malondialdehyde increased significantly ($p<0.001$) according to the duration of the clamp, especially at the 90th minute during hyperglycaemia (36,32±6,12 vs 52,60±7,45) in comparison with the control group. 3. Significantly ($p<0.001$) higher levels of glutathione were found in comparison with the control group during euglycaemia (1,47±0,05 vs 2,80±0,07 and 1,48±0,10 vs 2,66±0,10) and hyperglycaemia (1,61±0,19 vs 2,53±0,35 and 1,49±0,23 vs 2,58±0,07). 4. No significant changes in M-value were found in comparison with the control group in both modifications of the clamp. **Conclusions:** The short-term exogenous hyperinsulinaemia reduced the production of reactive oxygen species (ROS) during hyperglycaemia in an animal model compared with the control group.

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ANTIOXIDANT VITAMINS SUPPLEMENTATION AMELIORATES SERUM LIPID PROFILE AND PARAOXONASE ACTIVITY IN DIABETIC RABBITS

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Background and Aims: Oxidative stress has been recently implicated in the pathogenesis of vascular complication in diabetes and HDL-associated paraoxonase (PON) seems to play a major role in the protection of LDL against peroxidation. The object of the study was to assess the impact of antioxidant vitamins supplementation (AVS) on serum lipid profile and PON activity in diabetic rabbits. **Materials and Methods:** Male chinchilla rabbits were rendered diabetics by i.v. injection of dithizone (35 mg/kg b.w.). Control rabbits (C) were received vehicle alone. In a week after dithizone injection D, were randomly allocated into two groups given AVS (α -tocopherol 100 mg+vitamin C 200 mg/kg b.w./day per os) and placebo (diabetic control - DC) for 3 month. Fasting blood samples were used for glycemia, IRI, lipid profile, conjugated dienes (CD), and NEFA levels determination. Serum paraoxonase (PON) activity was evaluated using paraxon as substrate. **Results:** AVS improved glucose control in diabetic rabbits reducing basal hyperglycaemia (8.3±0.2 vs 15.6±2.7 mmol/l in DC, $p<0.01$) and increasing plasma insulin 1.3-fold ($p<0.05$) as compared to DC. AVS produced significant decrease both in TG and LDL-C (by 28 % and 27 %, $p<0.05$ vs DC). Following AVS decrease in TC (13 %, $p<0.05$) and NEFA (42 %, $p<0.02$) were also observed. In addition after AVS HDL-C was enhanced by 40 % ($p<0.01$) in comparison with DC. Moreover, AVS attenuated lipid peroxidation decreasing serum DC levels 2-fold and augmented PON activity (59.8±0.9 vs DC: 37.6±2.2 U/l, $p<0.02$; C: 81.0±2.1 U/l). **Conclusions:** AVS is possessed of favourable effect on atherogenic risk factors in diabetic rabbits through improving glycemic control and lipid profile, attenuating lipid peroxidation and increasing antioxidative defences. Using AVS may be beneficial in prevention of vascular complication related to diabetes.

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EFFECTS OF STOBADINE TREATMENT ON PENTOSE PHOSPHATE PATHWAY, GLUTATHIONE-DEPENDENT ENZYMES, SUPEROXIDE DISMUTASE, AND LIPID PEROXIDATION IN THE BRAIN OF DIABETIC RAT; COMPARISON WITH VITAMIN E. Ç. Karasu¹, N. Ulus², A. Avcı³, M. Sahilli¹, O. Canbolat⁴, G. Ozansoy¹, N. An¹, M. Stefek⁴, S. Stole⁴ and A. Gajdosik⁴, Departments of Pharmacology and Biochemistry of Faculty of Pharmacy¹ and Medicine^{2,3}, Hacettepe^{2,3} and Ankara^{1,3} Universities-Turkey, and Slovak Academy of Sciences⁴-Slovak Republic.

Backgrounds and Aims: Because oxidant stress may exacerbate some complications of diabetes mellitus, this study investigated the effects of 10 weeks treatment with stobadine (ST, 24.7 mg/kg/day, i.o.), a novel pyridindole antioxidant, and vitamin E (vitE, 400-500 IU/kg/day, i.o.), alone or in combination with each another on the enzymes related to pentose phosphate pathway and glutathione metabolism, superoxide dismutase (SOD) activity, and lipid peroxidation (MDA) in 10 weeks streptozotocin (55 mg/kg, i.p.)-diabetic (D) rats and age-matched control (C) rats. **Materials and Methods:** Glucose-6-phosphate dehydrogenase (G6PDH), 6-phospho-gluconate dehydrogenase (6PGDH), glutathione reductase (GR), glutathione peroxidase (GPx), glutathione-S-transferase (GST) (each were expressed as U/g protein), SOD (U/mg protein) and MDA (nmol/mg protein) were measured by spectrophotometric methods.

Results:	C (n=8)	C+ST+vitE (n=6)	D (n=11)	D+ST (n=13)	D+vitE (n=11)	D+ST+vitE (n=11)
G6PDH	15.3±1.1	15.7±1.1	22.5±0.2*	22.8±1.1*	25.8±0.7*	18.9±0.8*
6PGDH	6.58±0.5	6.59±0.7	6.51±0.5	6.42±0.5	7.03±0.5	6.74±0.9
GR	15.1±0.6	15.7±1.0*	11.9±0.5*	18.1±0.5*	17.1±0.8*	17.9±0.6*
GPx	6.62±0.5	6.60±0.5*	14.1±1.6*	9.67±0.6*	7.72±0.8*	6.90±1.0*
GST	88.6±2.3	110.6±9.5*	70.7±5.5	96.7±3.8*	97.6±8.2*	104.4±8.3*
SOD	41.2±2.8	41.9±2.9	40.3±1.8	40.3±3.7	43.2±4.9	46.0±3.7
MDA	0.39±0.10	0.29±0.01	1.36±0.2*	0.52±0.05*	0.45±0.10*	0.40±0.06*

Mean ±SEM; * $p<0.05$ vs C; ** $p<0.05$ vs D; ANOVA

Diabetic rats untreated or treated with ST or vitE showed increased G6PDH; this was reduced with combined therapy. 6PGDH or SOD was not changed by diabetes or by treatment. A decrease in GR of diabetic rats was prevented by ST treatment either alone or with vitE. Diabetes caused an increase in GPx and MDA, this was prevented by both antioxidants. ST or vitE stimulated GST activity either in control or in diabetic rats. The combined effect of ST and vitE on GST, GPx and MDA was gently greater than that of ST or vitE alone. **Conclusion:** Results indicate that combined therapy with ST plus vitE is effective to prevent diabetes-induced abnormalities in pentose phosphate pathway and glutathione redox cycle activity and associates with protection against an oxidant-induced brain injury.

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START OF INSULIN TREATMENT AND PLASMA ANTI-OXIDANTS IN NEW ONSET INSULIN REQUIRING DIABETES MELLITUS

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Background and Aims: Hyperglycaemia plays an important role in oxidative stress, which is probably involved in late diabetic complications. Oxidative stress can be evaluated indirectly by measuring anti-oxidant levels in plasma. **Materials and methods:** 15 Patients with new onset diabetes mellitus, requiring acute insulin treatment, aged 20-67 years, mean HbA1c 14.3 ± 3.65%, without taking vitamin supplements, were included subsequently. We assessed plasma α -tocopherol, retinol, β -carotene, uric acid and ceruloplasmin, before and monthly during six months of intensive insulin treatment. **Results:** Before insulin treatment α -tocopherol (vitamin E) levels in plasma were not different compared to non diabetic subjects (33.5 ± 12.1 vs 32.2 ± 0.8 μ mol/L). After insulin treatment α -tocopherol decreased significantly as shown in the table below:

	Before treatment	1 month insulin	3 months insulin
HbA1c, %	14.3 ± 3.65	9.6 ± 1.98*	5.9 ± 0.1*
Tot chol, mmol/L	5.44 ± 1.09	4.79 ± 0.89*	4.80 ± 1.17*
α -toco, μ mol/L	33.5 ± 12.1	28.11 ± 6.85*	26.6 ± 7.03*
α -toco/ tot chol, μ mol/ mmol	6.38 ± 1.32	5.78 ± 0.93*	5.69 ± 1.02 *

(Mean ±SD, * $p<0.05$ vs baseline, α -toco= α -tocopherol, tot chol=total cholesterol)

After six months α -tocopherol was still decreased compared to baseline levels (29.6 ± 7.4 but not significantly $p<0.80$). No significant changes in other plasma antioxidants were found. **Conclusion:** Insulin treatment and/or improved metabolic control causes during the first months a significant decrease of vitamin E plasma levels in new onset insulin requiring diabetes mellitus. May we conclude: *Insulin behaves as a pro-oxidant?*

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STUDIES ON PLASMA LIPIDS AND OXIDIZABILITY IN DIABETES TYPE 1 COMPLICATED BY NEPHROPATHY

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Aim: Mortality due to ischaemic heart disease in IDDM complicated by nephropathy is at least 9 times higher than in IDDM without albuminuria. One of the important pathogenetic mechanisms in this phenomenon probably are the quantitative and qualitative changes in plasma lipids and in the oxidizability of the LDL fraction.

Methods: In 3 groups of the IDDM patients: without microalbuminuria (20 cases), with microalbuminuria (20 cases), and with overt nephropathy (20 cases) the plasma levels of the total cholesterol, LDL-cholesterol, total triglycerides and the content of triglycerides in LDL fraction was assessed. Also the oxidizability under action of Cu²⁺ ions of the separated by ultracentrifugation (Beckman) LDL fraction in the pharmacokinetic system was studied by continuous registration of absorbency with the Shimadzu apparatus and the special computer program. **Results:** When compared in the respect of the all parameters under study all 3 groups differed in a statistically significant manner. LDL oxidizability was lowest in the IDDM group without microalbuminuria, higher in microalbuminurics and the highest in overt nephropathy. **Conclusion:** Abnormalities in plasma lipids and the increase of the oxidizability of LDL fraction were parallel to the intensity of albuminuria and the diabetic insult to the kidneys. They have atherogenic character and should become the object of the therapeutic limitations.

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The hypolipidemic potential and insulin sensitizing effect of n-3 PUFAs and TTA is related to their ability to stimulate peroxisomal fatty acid oxidation

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Background: The hypotriglyceridemic effect of marine fish oil (FO) and tetradecyl-thio-acetic acid (TTA) is associated with a decrease in adipose tissue mass and improvement of insulin action. The peroxisomal fatty acid /FA/ oxidation is a key player in metabolism of long chain FA and, therefore, may control triglyceride (TG) metabolism. Thus, aim of this study was to elucidate a possible role of the peroxisomal FA oxidation for the whole body insulin action. The effects of FO and TTA on the activity and gene expression for the key enzyme of peroxisomal oxidation of FA, i.e. acyl-CoA oxidase /AOX/ were, therefore, studied in the high fat (HF) diet-induced insulin resistance of rats. **Methods:** Male Wistar rats were fed for 21 days a high (70-cal%) fat (HF) diet. Two groups of them received a fish oil (HF/FO, 10 wt.) or TTA (HF/TTA, 150 mg/kg/day) enriched HF diet. The AOX enzyme activity was measured radiometrically in liver and skeletal muscle. Northern blots were used for evaluation of the gene expression. **Results:** N-3 PUFA supplementation of the HF diet lowered plasma triglycerides /TG/ by 70%. Liver AOX activity rose by more than 140% (HF: 4.2±0.4, HF/FO: 10.2±0.1 pmol.mg⁻¹.min⁻¹, p<0.05). This was accompanied by an 83 % increase in the mRNA level for AOX (HF: 0.6±0.03, HF/FO: 1.1±0.03, AU, p<0.05). Very similar effects of n-3 PUFAs were found for both AOX parameters in skeletal muscles. The *in vivo* insulin resistance /euglycemic hyperinsulinemic clamp/ of rats fed the HF diet was ameliorated by the n-3 PUFA supplement (HF: 15.7±0.4, HF/FO: 22.3±0.7 mg.kg⁻¹.min⁻¹, p<0.05). TTA supplemented HF diet led to an 81 % decrease in plasma TG. Liver AOX activity rose by 5-fold (HF: 4.2±0.4, HF/TTA: 24.5±2.1, pmol.mg⁻¹.min⁻¹, p<0.05) and the AOX mRNA level went up by 136%. TTA stimulated the activity and gene expression for AOX in the skeletal muscle as well. Also in this case, TTA consumption normalized the whole body insulin action. **Conclusions:** a) both investigated hypolipidemic compounds stimulated peroxisomal FA oxidation, what b) may be related to their positive influence on the overall insulin action in the HF diet-induced model of insulin resistance. c) The more pronounced effect of TTA on the peroxisomal FA oxidation in the liver points toward a higher hypolipidemic potential of TTA as compared to n-3 PUFAs. d) Nevertheless, such difference was not found in the skeletal muscle.

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Insulin and C-Peptide

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DIP1 and SF2 p32 are novel interacting proteins of the atypical protein kinase C zeta

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Background and Aims: The protein kinase C (PKC) family consists of 12 isoforms which are classified into three subfamilies (classical, novel and atypical) based on their structure and cofactor regulation. As serine/threonine kinases they play crucial roles in many different signal transduction pathways. The atypical protein kinase C zeta is a downstream target of the insulin/IRS-1/PI 3-kinase/PDK-1 cascade and is involved in insulin-stimulated protein synthesis and translocation of GLUT4 to the plasma membrane. Overexpression of atypical PKC isotype specific interacting protein (ASIP), the human homolog of *C.elegans* par-3, inhibits insulin-stimulated glucose uptake. Until now, however, little is known how PKC zeta mediates insulin specific signalling. The aim of the study was to further our understanding of PKC zeta downstream events and to find additional interacting proteins.

Materials and Methods: A human skeletal muscle cDNA library was screened for PKC zeta regulatory domain interacting proteins using the yeast two-hybrid system. To verify the interactions found in yeast, expression vectors encoding epitope tagged forms of the identified proteins and of PKC zeta were co-transfected in 293 cells. The localization of the proteins in overexpressing NIH/3T3 cells was characterized with immunofluorescence.

Results: Using the amino-terminus of PKC zeta as a bait in yeast two-hybrid system DIP1 and SF2 p32 were identified as interacting proteins. The interactions were verified in 293 cells coexpressing the complete PKC zeta protein and DIP1 or SF2 p32. To further demonstrate the relevance of the interaction, colocalization of the proteins was demonstrated by immunofluorescence.

Conclusions: Although at the moment there is no hint that the described interactions play a role in the insulin/IRS-1/PI 3-kinase/PDK-1 cascade, our results may provide new insights into insulin dependent PKC zeta signal transduction. The nuclear protein DIP1 is involved in gene expression and differentiation. SF p32 protein is a binding protein for nuclear pre-mRNA splicing factor SF2 and numerous other cellular proteins. By interacting with DIP1 and SF2 p32, PKC zeta could directly effect gene expression and pre-mRNA splicing.

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C-PEPTIDE STIMULATES Na,K-ATPase THROUGH PKC PATHWAY IN THE MEDULLARY THICK ASCENDING LIMB.

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Background and Aims: the connecting peptide (C-peptide), a cleavage product of insulin secretion, exerts several biological effects, in part through stimulation of Na,K-ATPase activity. The medullary thick ascending limb (MTAL) Na,K-ATPase is the target of several hormones and neurotransmitters. This study was therefore designed to evidence a specific physiological effect of C-peptide on Na,K-ATPase activity in MTAL, and determine the signaling pathway involved. **Materials and Methods:** isolation of MTAL segments was obtained by microdissection from collagenase-treated kidneys on male Wistar rats. The hydrolytic activity of Na,K-ATPase (V_{max} conditions) and ouabain-sensitive rubidium (86Rb) uptake (rate limiting [Na]) were determined on permeabilised and intact tubules respectively. Cell surface expression of Na,K-ATPase was determined by western blotting after a biotinylation and streptavidin-precipitation assay. **Results** show that biosynthetic rat C-peptide (II) dose-dependently stimulated Na,K-ATPase activity with a threshold close to 10⁻⁹ M while the maximal effect was observed with 10⁻⁷ M. Kinetic studies indicate that C-peptide (10⁻⁷ M) time-dependently stimulated Na,K-ATPase activity after 5 min and reached a plateau after 10 min while longer incubation time (15-60 min) did not result in further stimulation. Incubation of tubules with 10⁻⁷ M C-peptide at 37 °C for 15 minutes stimulated Na,K-ATPase activity (3067±278 vs 2182±136 pmol Pi.mm⁻¹.h⁻¹, p<0.01) and 86Rb uptake (21.8±1.6 vs 16.7±1.32 pmol Rb.mm⁻¹.min⁻¹; p<0.05) to the same extent, i.e. about 35 % of the control. The effects of C-peptide on Na,K-ATPase activity were completely abolished by GF209203Y, a specific inhibitor of PKC. C-peptide induced a translocation of PKC alpha isoform from soluble to particulate compartment. In contrast, C-peptide did not alter adenylate cyclase and cell surface expression in MTAL. **Conclusions** : our study provides evidence that C-peptide stimulates Na,K-ATPase activity within the physiological range of concentration. This effect relies on an increase in Na,K-ATPase turnover rate mediated by PKC alpha activation. Altogether, these results are consistent with a contribution of C-peptide to the stimulation Na,K-ATPase activity during non-fasting periods in MTAL.

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Ceramide induced inhibition of PKB signalling in skeletal muscle cells: a role for atypical PKCs?

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Background and Aims: One of the main physiological responses to insulin is the stimulation of cellular glucose uptake in skeletal muscle. This stimulation is mediated in a phosphatidylinositol 3-kinase (PI3K)-dependent manner, and increasing evidence suggests that activation of Protein Kinase B (PKB) which lies downstream of PI3K is also a key component of the signalling cascade that induces an increase in glucose uptake. We have recently shown that the insulin mediated activation of PKB and glucose transport are substantially reduced by the sphingomyelin lipid derivative ceramide which has been implicated in the pathogenesis of insulin resistance. The precise mechanisms by which ceramide attenuates insulin action are unclear. However, recent work has suggested that ceramide activates atypical PKC zeta/lambda, and that this kinase can negatively regulate PKB signalling. In this study we have assessed whether inhibition of PKC isoforms using selective inhibitors (Ro 31-8220, GF 109203X) can prevent the hormonal loss in the activation of PKB and glucose transport by ceramide in cultured muscle cells. **Materials and Methods:** PKB activity and phosphorylation state was assessed in L6 myotubes using a synthetic peptide substrate and phospho-specific antibodies, respectively. Glucose uptake was assayed using [3H]-2DG as a tracer. Cells were treated with ceramide (100µM, 2h), and/or insulin (100nM 10min) in the absence or presence of PKC inhibitors prior to assaying PKB activity and 2DG uptake. **Results:** Insulin induced a 15-fold increase in PKB activity and glucose uptake, which was lost completely upon prior incubation of cells with ceramide. Both Ro 31-8220 and GF 109203X prevented the ceramide-induced loss in PKB activation in a dose-dependent manner. The effect of both compounds was evident at concentrations that reportedly inhibit the activation of atypical PKCs (i.e PKC zeta/lambda). This effect was not observed at concentrations that inhibit conventional and novel PKCs. At the effective concentration Ro 31-8220 (5 µM) also prevented the loss in the hormonal stimulation of glucose uptake. **Conclusions:** These findings indicate that the role of ceramide in the development and progression of insulin resistance in skeletal muscle may arise through its ability to activate atypical PKC zeta/lambda, which may act negatively on insulin signalling to end point responses such as glucose transport.

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INSULIN RECEPTOR INTERNALIZATION AND INTRACELLULAR SORTING ARE REGULATED BY PROTEIN KINASE C-ZETA AND PROTEIN KINASE B/AKT.

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Background and Aims: Insulin receptor (IR) tyrosine kinase activity is essential for insulin internalization. However, the molecular mechanisms controlling IR endocytosis and further intracellular sorting remain poorly defined.

Materials and Methods: We have addressed this issue by following radiolabeled insulin internalization and sorting in L6 cells expressing human insulin receptor (L6hIR), which have been treated or not with selective pharmacological inhibitors.

Results: We found that insulin internalization was inhibited by about 50% in cells treated with wortmannin (WM) or LY294002, which block phosphatidylinositol, 3 kinase (PI3K). Internalization was not affected by bisindolylmaleimide and PD98059, which inhibit conventional protein kinase C (PKC) isoforms and MEK/MAPK, respectively. WM and LY294002 inhibited insulin retroendocytosis by 70% and insulin degradation by only 20%, thus impairing normal intracellular sorting. The effect of PI3K inhibitors on insulin internalization was mimicked by transient transfection of either a dominant negative PKCzeta mutant and PKCzeta antisense oligonucleotides. However, both insulin degradation and retroendocytosis were reduced by 50% when PKCzeta was blocked. Conversely, overexpression of wt-PKCzeta in L6hIR cells increased by >2-fold insulin internalization, degradation and retroendocytosis. At variance with PKCzeta, expression of wt and constitutively active Akt/PKB had no effect on insulin internalization and increased by 2.5-fold insulin retroendocytosis with minor changes of insulin degradation.

Conclusion: Thus, PI3K controls different steps within insulin endocytic itinerary. PKCzeta appears to mediate PI3K effect on insulin internalization while Akt/PKB directs intracellular sorting toward retroendocytosis.

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Impaired insulin signal transduction and glucose transport in the type 2 diabetic heart

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Background and aim: The relationship between insulin resistance and cardiovascular risk is well established but, little is known about the molecular mechanisms involved and, in particular, about alterations in the insulin response which may underlie the abnormalities observed in diabetic heart. The purpose of this study was to determine the response to insulin and initial insulin signaling steps in the heart of a polygenic model of type 2 diabetes, the GK rat.

Materials and methods: We measured the initial steps of the insulin signaling pathway after insulin infusion via the caudal vena cava as well as 3H glucose uptake after insulin stimulation in hearts from the male Wistar and spontaneously diabetic GK rats.

Results: Insulin injected into the caudal vena cava caused maximal tyrosine phosphorylation of myocardial proteins within 90 s. We found a 33% decrease in insulin receptor b content ($p < 0.0001$) in the GK rat heart, associated with 41% lower ($p < 0.02$) insulin-stimulated tyrosine phosphorylation of the insulin receptor b subunit compared with control hearts. Insulin-stimulated tyrosine phosphorylation of IRS-1 and its association with the p85 subunit of PI 3-kinase were reduced in GK rat heart by 54% ($p < 0.01$) and 57% ($p = 0.02$) respectively, which correlated with a 41% decrease in IRS-1 content ($p < 0.001$) in the GK rat heart. However, protein level and insulin-stimulated phosphorylation of Akt1/PKBa and MAPK1/2 were the same in both GK and control rat hearts. Measurement of 3H glucose uptake showed that the GK rat hearts had basal glucose uptake similar to the control rat hearts but insulin-stimulated glucose uptake was 20% lower ($p < 0.05$) in the GK rat hearts compared with normal rat hearts. A 28% decrease in total GLUT4 protein content ($p < 0.01$) was found in the GK rat heart, with normal total GLUT1 protein.

Conclusion: We conclude that insulin-stimulated glucose uptake is reduced in type 2 diabetic heart due to impaired insulin signal transduction and a loss of GLUT4 protein.

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GLUT5 expression and fructose transport in rat adipocytes: effects of age and insulin resistance

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Background and Aims: We have previously shown that rat adipocytes express the GLUT5 fructose transporter in the plasma membrane. However little is known about the exact role of this transporter and the extent to which fructose transport is important for the metabolism of this sugar in adipose tissue. In this study we have investigated the effects of age and insulin resistance on transporter expression and fructose metabolism in adipose tissue of Zucker rats. **Materials and Methods:** Young (age 5 weeks) and mature (age 15 weeks) lean and obese Zucker rats were used in this study. Adipose tissue was excised and processed for either isolation of subcellular membrane fractions (plasma membrane, PM; light microsomes, LDM; and heavy microsomes, HDM), or used in studies of fructose uptake, lactate production, and lipid synthesis using [14C]-labelled fructose. **Results:** (1) Effects of age: Analysis of GLUT5 protein expression in adipocytes isolated from young and mature lean animals revealed a 78% decrease in transporter expression in the mature compared to the young lean rats. This loss in GLUT5 expression was associated with a 50% reduction in fructose uptake between the two age groups. (2) Effects of insulin resistance: The young obese animals presented increased blood insulin compared to their lean littermates (5.18 ± 0.94 ng/ml versus 1.95 ± 0.53 ng/ml ($p < 0.05$), respectively) but did not show any significant increase in blood glucose indicating that these animals were insulin resistant at 5 weeks. Immunoblot analysis revealed a 1.8-fold increase in GLUT5 expression in the PM of the young obese animals compared to corresponding lean controls. This up-regulation in GLUT5 was accompanied by an increase in fructose uptake, lactate production and lipid synthesis by ~3-4 fold. As with the lean animals, obese animals showed an age-related loss in GLUT5 expression (-84%), and although differences in GLUT5 expression were observed between lean and obese at 5 weeks, this was not observed at 15 weeks. **Conclusions:** The findings provide further evidence that GLUT5 is responsible for fructose uptake in adipose tissue, and that modulation of its expression results in associated changes in the metabolism of fructose in response to ageing and insulin resistance.

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Chronic Angiotensin II Receptor Antagonism Enhances Skeletal Muscle GLUT-4 Protein Expression and Glucose Transport Activity in the Obese Zucker Rat. T.R. Kinnick, S. Jacob, M.K. Teachey, M. Krekler, and E.J. Henriksen. Department of Physiology, University of Arizona College of Medicine, Tucson, AZ USA; Department of Endocrinology, Eberhard-Karls University, Tübingen, Germany; and Bristol-Myers Squibb, Munich, Germany.

Background and Aims: Recent evidence supports a role of angiotensin II (ATII) in the pathogenesis of skeletal muscle insulin resistance. In the present study, the effects of chronic administration of an ATII (selective AT1-subtype) receptor antagonist (irbesartan) on glucose tolerance, insulin action on skeletal muscle glucose transport, and muscle GLUT-4 glucose transporter protein expression were assessed in the insulin-resistant obese Zucker (fa/fa) rat.

Materials and Methods: Female obese Zucker rats were treated with 50 mg/kg irbesartan by gavage for 21 consecutive days. An oral glucose tolerance test (OGTT; 1 g/kg; 120 min) and determination of insulin-stimulated glucose transport activity (2-deoxyglucose uptake; 1 mM) in isolated epitrochlearis and soleus muscles were then completed. GLUT-4 protein levels in muscle and heart were also measured.

Results: Chronic ATII receptor antagonism was associated with an 18% reduction ($p < 0.05$) in heart mass and a 14% diminution ($p < 0.05$) of fasting plasma glucose. The glucose and insulin responses (areas under the curve (AUC)) during the OGTT were reduced by 19% and 21%, respectively (both $p < 0.05$), in the irbesartan-treated group compared to vehicle-treated controls. The glucose-insulin index (the product of the glucose and insulin AUCs during the OGTT) was reduced by 34% ($p < 0.05$) in the irbesartan-treated obese group, indicative of an increase in whole-body insulin sensitivity. Insulin-mediated glucose transport was significantly elevated in both type I soleus (73%) and type IIb epitrochlearis (32%) muscles from irbesartan-treated animals. The irbesartan-induced modification of whole-body insulin sensitivity was significantly correlated with the increased insulin-mediated glucose transport in both epitrochlearis ($r = -0.677$, $p < 0.05$) and soleus ($r = -0.892$, $p < 0.05$) muscles. Chronic ATII receptor antagonism was also associated with small, but significant ($p < 0.05$), increases in GLUT-4 protein expression in the soleus (22%), plantaris (20%), and myocardium (15%).

Conclusions: In insulin-resistant obese Zucker rats, chronic antagonism of ATII receptors (AT1-subtype) lowers fasting plasma glucose and improves glucose

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Evidence for a common step in arsenite- and insulin-induced glucose uptake in 3T3-L1 adipocytes

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Background and Aims: Insulin and stress-related stimuli like hyperosmotic shock and muscle contraction stimulate GLUT4 mediated glucose uptake in muscle cells and adipocytes. Most studies indicate that these stimuli induce the translocation of the main insulin-responsive glucose transporter GLUT4 to the plasma membrane, suggestive of convergence with the insulin-induced signal transduction pathway. Remarkably, in contrast to insulin, glucose uptake induced by these stimuli was found to be independent of PI-3' kinase activation. The mechanism underlying glucose uptake mediated by these stimuli is only partially understood. To analyse in detail the mechanism of stress- induced glucose uptake, we have compared the signaling intermediates that are activated by arsenite, an inducer of chemical stress, and insulin.

Materials and Methods: In this study we used 3T3-L1 adipocytes as a tool to compare stress- and insulin-induced glucose uptake. Apart from subcellular localisation of GLUT4 upon stimulation with either arsenite or insulin, we investigated the activation of several components of the insulin induced signal-transduction pathway, as well as the effect of pharmacological- and peptide-inhibitors on arsenite-, osmotic shock and insulin-induced glucose uptake.

Results: Like insulin, arsenite stimulates basal glucose uptake, concomitant with translocation of GLUT4 towards the plasma membrane. However, arsenite did not activate early steps of the Insulin Receptor signal transduction such as Tyrosine-phosphorylation of IRS-1 or -2 and activation of PI-3' kinase. Furthermore, arsenite induced glucose uptake was insensitive to inhibition of PI-3' kinase by wortmannin. However, we did find that Ro31-8220 was capable of inhibiting insulin- as well as arsenite- or osmotic shock induced glucose uptake. Ro31-8220 inhibited glucose uptake with an IC50-value suggestive of the involvement of atypical PKC-lambda in this process. Moreover, a myristoylated peptide-inhibitor that has been shown to act as a PKC-lambda pseudosubstrate also inhibited insulin- as well as arsenite induced glucose uptake.

Conclusions: These data indicate that arsenite activates a signaling pathway downstream of PI-3' kinase in the insulin signal transduction pathway that is sensitive to commonly used inhibitors for atypical PKC-lambda.

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Disordered Kinetics of Exogenous Insulin in Type 2 Diabetes: A Study of Insulin Volume of Distribution and Metabolic Clearance in Diabetic and Non-Diabetic Subjects

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Background and Aims: There is evidence in both type 2 diabetes and normoglycaemic insulin resistant states that the metabolic clearance rate (MCR) of insulin is slower than in insulin sensitive individuals. The importance of this is not fully understood. In addition, there are differences between diabetic and non-diabetic subjects in the apparent volume of distribution (Vd) of insulin, but the data are not consistent and appear conflicting. We aimed to clarify the kinetics of exogenous insulin under fasting conditions in relation to insulin sensitivity.

Materials and Methods: We have performed a series of short insulin tolerance tests using 0.02U/kg of intravenous human actrapid in 16 patients with type 2 diabetes and 12 normoglycaemic control subjects. All diabetic patients were controlled on diet alone and were not receiving any antihypertensive medication. Insulin sensitivity was measured by the rate of decrease in plasma glucose concentration over 15 minutes. Specific insulin was measured by an ELISA with negligible cross reactivity with all insulin precursors. Non-linear regression was used to fit a biexponential curve to the insulin decay profile, described by $y = A \cdot e^{-(Bt)} + C \cdot e^{-(Dt)} + E$, assuming a two compartment model. A frequent sampling protocol ensured accuracy of the y intercept and integration of the regression plot. Non-parametric analysis was used to assess significance of the data.

Results: Diabetic subjects had greater fasting hyperinsulinaemia (median 4.95 pmol/l [interquartile range 3.41–10.38] v 1.95 [1.37–2.28]; $p < 0.05$), and hyperglycaemia (6.97 mmol/l [5.75–8.07] v 5.25 [5.0–5.45]; $p < 0.05$), and were more insulin resistant than controls. The Vd of insulin was less in diabetes than controls (555.2 ml/kg [359.1–740.5] v 924.7 [682.5–1608.6]; $p < 0.05$). The MCR of insulin was slower in diabetes (206.9 ml/min/kg [124.3–241.0] v 531.3 [257.2–694.7]; $p < 0.05$). There was no identifiable difference in insulin mean residence time, disposal rate or half life between groups.

Conclusions: Under fasting conditions, the Vd of exogenous insulin in type 2 diabetes is decreased compared to the insulin sensitive state. We have confirmed that insulin MCR is slower in type 2 diabetes. Impaired insulin delivery to target sites may contribute to peripheral insulin resistance. Similarly, insulin degradation occurs at target sites and impaired delivery also may underlie slower clearance rates.

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IMPORTANCE OF GLU 27 FOR C-PEPTIDE BINDING TO CELL MEMBRANES

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Background and Aim. Besides its role in facilitating proinsulin folding C-peptide has been shown to exert cellular effects. When given to patients with type 1 diabetes, C-peptide stimulates blood flow and improves renal and nerve function. Binding of C-peptide in the nanomolar concentration range to cell membranes has been demonstrated using fluorescence correlation spectroscopy (FCS). In this study we examine the structural requirements for cellular binding of C-peptide and its pentapeptide.

Methods and Materials. Binding of 5 nM tetramethyl rhodamine labeled human C-peptide (RhCP) to human renal tubular cells in primary culture and its displacement by different peptides was studied using FCS. Competitive displacement of bound RhCP by the C-terminal peptide E₂₇GSLQ₃₁, derivatives thereof in which all residues were sequentially replaced by Ala, and fragments of C-peptide was studied.

Results. Bound RhCP was displaced to 80-90% within 60 min after addition of 5 μM unlabeled C-peptide or EGSLQ. AGSLQ did not elicit detectable displacement. EASLQ gave rise to approximately 80% competitive displacement and incubation with EGALQ, EGSAQ and EGSLA resulted in intermediate displacement values of 28%, 20% and 42%, respectively. Incubation with 5 μM Glu elicited 50% displacement, while G₂₈SLQ₃₁, E₁₁LGGGPGAG₁₉, or (1-26) C-peptide all failed to elicit displacement of bound RhCP. It is concluded that Glu 27 is of critical importance for C-peptide's binding to cellular targets.

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C-PEPTIDE AND ITS ANALOGUES STIMULATE INTRACELLULAR Ca^{2+} CONCENTRATIONS

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Background and Aims: Proinsulin C-peptide shows specific binding to cell membranes followed by activation of a G-protein coupled membrane receptor and stimulation of Ca^{2+} dependent signaling pathways. This study aims to further define the ability of C-peptide and its analogues to increase intracellular Ca^{2+} levels.

Methods and Materials: Human renal tubular cells in primary culture were exposed to fura-2/AM. (2 μM , 35 min) The changes in intracellular Ca^{2+} concentrations, $[\text{Ca}^{2+}]_i$, presented as the 340/380 nm fluorescence ratio were measured by microfluorimetry after exposure of the cells to 10^{-9} – 10^{-7} M C-peptide, its C-terminal pentapeptide (human and rat) and derivatives of the pentapeptide obtained by stepwise substitution with Ala.

Results: Human C-peptide and its C-terminal pentapeptide ($\text{E}_{27}\text{GSLQ}_{31}$), but neither (1-26) C-peptide nor randomly scrambled C-peptide, elicited a significant rise in intracellular Ca^{2+} . EGALQ showed similar effects as the native pentapeptide, EALSQ and EGSLA had slightly lower effects and AGSLQ had approximately half of the native pentapeptides' effect. Preincubation of the cells with pertussis toxin (1 $\mu\text{g}/\text{ml}$, 3 h, 37°C) abolished the Ca^{2+} stimulatory effect of C-peptide and the pentapeptide. Both rat C-peptide and rat pentapeptide ($\text{E}_{27}\text{VARQ}_{31}$) elicited a Ca^{2+} rise similar to that for the corresponding human peptides. It is concluded that C-peptide and its C-terminal pentapeptide are equipotent in stimulating increments of intracellular Ca^{2+} , and that Glu 27 is of importance for this effect.

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Effect of C-peptide on Glucose Metabolism in Type 1 Diabetic Patients

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Background and Aims: C-peptide stimulate glucose transport in human skeletal muscle and increases glucose utilisation in STZ-induced diabetic rats in an NO-dependent manner. However, the effect of C-peptide on glucose metabolism in humans is still a matter of debate.

Materials and Methods: In a double blind, placebo controlled, two-way cross-over study we investigated the glucodynamic effects of an intravenous infusion of C-peptide in two different doses (2 pmol/kg/min for 90 min and 8 pmol/kg/min for another 90 min). Ten type 1 diabetic patients (6 male/4 female; aged 25 to 45 years; duration of diabetes 15 ± 10 years) participated in an euglycaemic glucose clamp (continuous insulin infusion of 0.2 mU/kg/min, blood glucose 5.5 mmol/l).

Results: C-peptide infusion resulted in an increase of serum C-peptide levels during the low infusion period from 0 to 0.58 ± 0.20 nmol/L [mean \pm SD] and to 2.3 ± 0.67 nmol/L the high infusion period. In comparison to the metabolic effect observed during the placebo infusion periods, the glucose infusion (AUC) necessary to keep blood glucose constant was lower during the low C-peptide infusion period (25.5 (6-73.5) vs. 69.0 (33.0-132.0) mg/kg/min; $p < 0.05$ [median (interquartile range)] and tended to be lower during the high infusion period (28.5 (10.0-103.5) vs. 88.0 (63.0-119.5) mg/kg/min; $p = 0.07$). Total glucose consumption during both C-peptide infusion periods was lower (79.5 (25.0-144.0) vs. 157.5 (75.5-251.0) mg/kg/min; $p < 0.05$). Serum insulin levels were not different during placebo and C-peptide infusion periods (12.6 ± 7.7 vs. 11.4 ± 7.7 $\mu\text{U}/\text{mL}$, n.s.).

Conclusions: In contrast to results obtained with isolated human muscle strips and in STZ-induced diabetic rats, our study could not demonstrate an activation of glucose metabolism during short term C-peptide supplementation in type 1 diabetic patients. Beside its well known vascular effects, C-peptide seem to have no stimulating effect on glucose metabolism in type 1 diabetic patients.

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Insulin and IGF-1 Receptors

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ROLE FOR SAM68 AS A DOCKING PROTEIN IN INSULIN RECEPTOR SIGNALING

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Background and Aims: The 68 kDa Src substrate associated during mitosis (Sam68) is an RNA binding protein with Src homology (SH) 2 and 3 domain binding sites. We have recently found that Sam68 is a substrate of the insulin receptor (IR) and that Tyr-phosphorylated Sam68 associates with the SH2 domains of p85 PI3K, in vivo and in vitro. In the present work we sought to study the association of Sam68 with the Ras-GAP pathway by assessing the interactions with the SH2 domains of GAP and the SH3 domains of Grb2.

Materials and Methods: We employed GST-fusion proteins of SH2 domains of GAP (N or C) and SH3 domains of Grb2 (N or C), recombinant Sam68 and purified IR for in vitro studies. In vivo studies of protein-protein interaction were assessed by co-immunoprecipitation experiments with specific antibodies anti-Sam68, GAP, Grb2, Sos and phosphotyrosine; and by affinity precipitation with the fusion proteins (SH2-GAP or SH3-Grb2).

Results: Insulin stimulation of HTC-IR cells promotes phosphorylation of Sam68 and its association with the SH2 domains of GAP. Sam68 is constitutively associated with the SH3 domains of Grb2 and it does not change upon insulin stimulation, but Sam68 is Tyr-phosphorylated and promotes the association of GAP with the Grb2-Sos complex. In vitro studies with fusion proteins showed that sam68 association with GAP and Grb2 are preferentially mediated by the C-terminal SH2 and SH3 domains of GAP and Grb2 respectively.

Conclusions: Sam68 is a substrate of the IR and may have a role as a docking protein in IR signaling, recruiting GAP to the Grb2-Sos complex, and in this way it may modulate Ras activity.

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Identification of a cis-acting element and a trans-acting factor which regulates the tissue specific insulin receptor gene in hepatocytes.

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Background and Aims: The insulin receptor is present in almost all cells and tissues, but its expression varies among the tissues. Especially in liver, muscle and fat cells, expression of insulin receptor is abundant. The liver is one of the major target organs of insulin and is important for glucose homeostasis. However, the mechanism of liver specific expression of the insulin receptor gene has not been well elucidated. In this study, we analyzed the tissue specific regulation of the insulin receptor gene in liver.

Materials and Methods: To determine the promoter region required for expression of the tissue specific insulin receptor gene in HepG2 cells, plasmids containing various lengths of the human insulin receptor promoter gene upstream of the chloramphenicol acetyl transferase (CAT) gene were constructed. The CAT activity of these plasmids were assayed in HepG2 cells. To clarify whether nuclear proteins can bind to the tissue specific cis-acting element of insulin receptor promoter gene, electrophoretic mobility shift assay (EMSA) were performed with nuclear extracts obtained from HepG2 cells and rat hepatocytes. Moreover, UV crosslink assay was performed to determine the molecular weight of the nuclear protein in HepG2 cells that binds to the tissue specific cis-acting element of insulin receptor promoter gene.

Results: In CAT assay with chimeric plasmids containing various deletions and insertions of the human insulin receptor promoter / CAT gene, a hepatocyte specific cis-acting element was identified nt -592 to -577 of the promoter region. In EMSA and UV crosslink assay, a 35kDa nuclear protein that bound to 5'-TCCCTCCC-3' (nt -588 to -581) sequence was identified in HepG2 cells and hepatocytes.

Conclusions: This novel nuclear protein identified in this study, may play an important role for tissue specific expression of the insulin receptor gene in the liver.

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17 β -ESTRADIOL DECREASES HUMAN INSULIN RECEPTOR mRNA LEVELS AND INSULIN RESPONSIVENESS IN U-937 PROMONOCYTIC CELLS.

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Background and Aims: Earlier studies of our laboratory and others have demonstrated regulation of insulin receptor (IR) gene expression in established cell lines by steroid hormones such as mineralocorticoids, glucocorticoids, progestins and androgens. Nonetheless, there are no studies demonstrating effects of estrogens on IR gene expression. For these reasons, in the present work we analyze the effect of 17 β -estradiol (E_2) on IR mRNA levels and insulin action in terms of glucose oxidation in U-937 human promonocytic cells. This cell line possesses estrogen receptor α and β as well as IRs and is considered to be a useful monocyte-like model to study the modulation of IR gene expression and insulin activity by diverse hormones. **Materials and Methods:** U-937 cells were treated for 24 h with increasing concentrations of E_2 (from 10^{-10} M to 10^{-6} M) and the IR mRNA levels determined by Northern blot assays. To assess the time-course of estrogen effect on IR mRNA levels, the cells were treated for 15, 24, 36 and 48 h with 10^{-9} M E_2 . Measurements of glucose oxidation were carried out in both untreated and cells treated for 24 h with 10^{-9} M E_2 in the absence/presence of increasing insulin concentrations (from 10^{-10} M to 10^{-7} M). **Results:** Northern assays showed that the levels of the two major IR-related mRNA species of approximately 11 kb and 8.5 kb in size present in these cells were decreased about 12% upon treatment with 10^{-10} M E_2 , reaching minimum values (30% decrease) with 10^{-9} M E_2 , and returning to near normal levels at higher concentrations of E_2 . This inhibition was also time-dependent reaching minimum values (34% decrease) 36 h after addition of E_2 . Basal values of glucose oxidation decreased by 24% after treatment for 24 h with 10^{-9} M E_2 . Insulin stimulated glucose oxidation in a dose-dependent manner in both treated and untreated cells. However, the maximal response at 10^{-7} M insulin was 69% lower in treated than in untreated cells indicating a decrease in responsiveness induced by E_2 . In conclusion, our results demonstrate for the first time an important role for E_2 as inhibitor of IR gene expression in U-937 human cells. This effect was accompanied by decreased insulin responsiveness of these cells.

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TRANSCRIPTIONAL ACTIVATION OF THE HUMAN INSULIN RECEPTOR GENE BY 1,25-DIHYDROXYVITAMIN D_3 .

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Background and Aims: We previously demonstrated that 1,25-Dihydroxyvitamin D_3 (1,25D $_3$) increased insulin receptor (IR) gene expression and insulin binding in U-937 human promonocytic cells. RNA stability resulted unaltered suggesting an effect on transcription. 1,25D $_3$ -treatment also caused an increase in the insulin responsiveness of these cells to both glucose transport and glucose oxidation. With these antecedents, in the present work we extend our previous studies analysing the possible existence of a direct transcriptional effect of 1,25D $_3$ on IR gene expression in U-937 cells. In addition, we study whether the potentiation of insulin action by 1,25D $_3$ is associated to the activation of the insulin signalling system at the level of phosphatidylinositol 3-kinase (PI3-kinase) in these cells. **Materials and Methods:** The -1819 to -271 promoter fragment of the IR gene cloned in Bgl II site of pCAT3M vector, kindly provided by Drs S.Y. Tsai and G. Elberg, was subcloned into the Bgl II site of a pGL2-basic vector (Promega) to create the reporter plasmid pIR(1.5)-GL2. Transient transfections with this and other positive and negative control plasmids were carried out by electroporation of 20×10^6 U-937 cells at 250 V, 960 μ F. After resting for 24 h the transfected cells were either untreated or treated for 24 h with 10^{-8} M 1,25D $_3$. Then, the cells were collected by centrifugation and the luciferase activity quantified. Glucose transport and glucose oxidation were measured at the concentration of insulin giving the maximal response: 10^{-8} M and 10^{-7} M respectively, and in the absence/presence of two concentrations of the PI3-kinase inhibitor wortmannin: 0.4×10^{-6} M and 10^{-6} M, using both untreated cells and cells treated for 24 h with 1,25D $_3$. **Results:** Treatment with 1,25D $_3$ caused a 1.8-fold transcriptional activation of the human IR gene. This activation seems to potentiate the maximal insulin response to both glucose transport (1.3-fold) and glucose oxidation (1.6-fold) in these cells. Wortmannin caused a greater inhibition of the insulin response to both glucose transport and glucose oxidation in 1,25D $_3$ -treated (0.2 – 0.5-fold) than in untreated cells (0.1 – 0.3-fold). This suggests an activation of PI3-kinase activity by 1,25D $_3$ that could mediate the potentiation of the insulin response.

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EFFECTS OF INSULIN GLARGINE ON CULTURED HUMAN SKELETAL MUSCLE CELLS: COMPARISONS WITH INSULIN AND IGF-1.

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Background and Aims: Insulin glargine (Lantus®) is a human insulin analog with a prolonged duration of action. This study compared the effects of insulin glargine (IG) with human insulin (HI) and IGF-1. **Materials and Methods:** Endpoints examined were receptor binding, glucose uptake, 3 H-thymidine uptake and activation of signalling molecules in differentiated Human Skeletal Muscle Cells (HSMC). HSMC were obtained from both healthy individuals (mean BMI 28.1 ± 1.3 kg/m 2 , fasting blood glucose (FBG) 4.9 ± 0.1 mM) and patients with type 2 diabetes (mean BMI 35.2 ± 2.1 kg/m 2 , FBG 8.1 ± 0.6 mM). **Results:** IG and HI were equipotent in their ability to compete for binding to the insulin receptor, whereas IGF-1 bound with only 1% of the affinity of insulin. IG and HI displaced IGF-1 from its receptor with very low affinity (IG < 0.5% affinity of IGF-1). The sensitivity of glucose uptake was greatest in response to IGF-1 and was lower and equal for IG and HI. Maximal stimulation of glucose uptake over basal levels was similar in response to all three ligands in non-diabetic cells, but higher in response to IGF-1 in diabetic cells. Stimulation of Akt phosphorylation was more responsive to IGF-1, compared with IG and HI, with sensitivities similar to those for stimulation of glucose uptake. Thymidine uptake was only marginally stimulated by IG and HI, compared to the potent response to IGF-1. MAPK phosphorylation was stimulated to a similar degree by all three ligands. **Conclusion:** We conclude that IG is equivalent to HI with respect to metabolic responses and does not display augmented mitogenic effects in skeletal muscle.

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THE SUBCELLULAR DISTRIBUTION OF INSULIN RECEPTOR SUBSTRATE-1 IS MODIFIED IN ADIPOCYTES FROM AGED RATS

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Background and Aims: Insulin receptor substrate (IRS) proteins are major substrate of the insulin receptor (IR), and it has been suggested that the intracellular location of IRS proteins is regulated in a way that may influence insulin action. In adipocytes, short term insulin stimulation triggers the release of IRS-1 from the low density microsome fraction into the cytosol. Then, defects within the subcellular localizations of IRS-1 protein may account for the development of insulin resistance. The aim of this work was to determine the subcellular localization and redistribution of IRS-1 after insulin stimulation of adipocytes in an insulin resistance state, as aging. **Materials and Methods:** Adipocytes from male Wistar adult (3-months) and old (24-months-old) rats were prepared from epididymal and retroperitoneal fat pads. After preincubation for 15 min, the adipocytes were stimulated with 80nM insulin for the indicated times. Cytosol and internal membranes (LDM and HDM) were isolated by differential centrifugation. Cytosolic protein (500 μ g) was immunoprecipitated with anti-IRS-1 and/or anti-PY antibodies. Immunoprecipitates and internal membranes (75 μ g) were analyzed by Western blotting with anti-IRS-1 and anti-PY antibodies. The bound antibodies were visualized using the ECL method, and bands intensities were quantitated by scanning densitometry of autoradiographs whose exposure was in the linear range. **Results:** Under basal conditions, the content of IRS-1 in the adipocytes of old rats is 15% lower and 10% higher in the internal membrane (IM) fraction and the cytosol, respectively, as compared to adult rats. Insulin promotes the translocation of IRS-1 from the IM fraction into the cytosol in both cases. However, the tyrosine phosphorylation of IRS-1 was decreased by 45% and 60% in the IM fraction and the cytosol, respectively, in response to insulin in old adult rats. **Conclusions:** In aging, the inappropriate accumulation of IRS-1 in the cytosol may disengage this protein from the IR, resulting in a significant reduction in tyrosyl-phosphorylated IRS-1 that may cause a state of insulin resistance.

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EFFECTS OF HUMAN INSULIN AND INSULIN ANALOGUES ON THE INSULIN RECEPTOR SIGNALING CASCADE

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Background and aims: Insulin glargine is a human insulin analogue with a prolonged duration of action. The aim of this study was to compare the effects of insulin glargine, human insulin (HI), and the insulin analogue Asp(B10) (which has been shown to have increased mitogenic effects via the insulin receptor, in various *in vitro* assay systems; rat adipocytes, rat-1 fibroblasts over-expressing the human insulin receptor and at the purified human insulin receptor). **Materials and Methods:** Insulin receptor binding was studied in rat-1 fibroblasts over-expressing the human insulin receptor and with purified human insulin receptor. Insulin receptor activation was assessed in rat-1 fibroblasts and in rat adipocytes. **Results:** Insulin glargine, HI and Asp(B10) insulin were shown to have similar insulin receptor association kinetics. Insulin glargine had slightly slower dissociation kinetics compared with HI, which is consistent with slightly decreased steady state binding affinity and metabolic activity. In contrast, Asp(B10) insulin had delayed dissociation kinetics and only partial dissociation over 60 minutes. Insulin receptor autophosphorylation kinetics were similar for insulin glargine and HI, but were delayed and prolonged in response to Asp(B10) insulin. A similar pattern was observed for the dephosphorylation kinetics of the insulin receptor and insulin receptor substrate IRS-1. Insulin glargine was similar to HI in terms of thymidine incorporation induced via insulin receptor activation in rat-1 fibroblasts. Asp(B10), however, induced a higher level of thymidine incorporation. **Conclusion:** The data presented here show that the characteristics of insulin glargine with respect to insulin receptor binding, activation of initial signalling events, metabolic activity and insulin receptor mediated thymidine incorporation are comparable with those of HI. In clear contrast, Asp(B10) insulin has slower receptor dissociation rates, prolonged phosphorylation of the insulin receptor and its substrates and increased potential for inducing mitogenic effects.

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PROLIFERATION AND IGF-BINDING PROTEINS PRODUCTION BY FETAL HEPATOCYTE IS CONTROLLED BY THE DIET OF THE DAM: AN *IN VITRO* STUDY

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Background: Maternal low protein diet (LP) during pregnancy in rats leads to low birth weight, less circulating IGF-I and less IGF-I and -II in pancreatic islets in the fetus. Insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) play a central role in growth and development. Both proteins are produced by liver cells and they are modulated by insulin and glucocorticoids, which are altered by the maternal diet. **Aim:** To study the effect of maternal LP diet on proliferation and IGFBPs production by fetal hepatocytes and the regulation by hormones. **Methods:** Pregnant Wistar rats were fed a control (C) diet (20% protein) or a low protein diet (8%) throughout gestation. At day 21.5, fetal hepatocytes were cultured during four days. Hormones were added from day 0 to day 4. Western Ligand Blot (WLB) using [¹²⁵I]-IGF-II as ligand and immunoblot using anti-IGFBP-1 and -2 antibodies were made on 24h culture media. The bands were quantified by densitometry in image analysis. DNA synthesis was estimated by DAPI (diamidinophenylindole) method and by the incorporation of [³H]-thymidine over 24 h periods. **Results:** WLB analysis revealed a band of 30-34 kDa (IGFBP-1 and/or 2) in C as well as in LP group which is however two times enhanced in the LP group (p<0.001). The immunoblots for IGFBP-1 (p<0.01) and IGFBP-2 (p<0.02) confirmed this pattern of increase in LP group. By contrast, maternal LP diet caused a significant 30% decrease in DNA synthesis (p<0.05). Addition of insulin at 200 and 1000 nM to the culture medium of C hepatocytes induced a decrease in the expression of the 30 kDa band and an increase in DNA synthesis (p<0.01), while 10 and 100 nM of dexamethasone markedly increased IGFBP production and reduced DNA synthesis (p<0.01). Addition of IGF-I and/or IGFBP-1 revealed that IGFBP-1 alone or in presence of IGF-I led to a significant decrease in DNA synthesis, mimicking dexamethasone effect. **Conclusion:** Maternal LP diet induces an overexpression in IGFBPs associated with an impaired DNA synthesis. These effects are modulated by insulin and glucocorticoids which have been found modified by maternal malnutrition. These results also suggest that the activity of these two hormones on fetal hepatocyte proliferation may involve the regulation of IGFBPs production.

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Glycogen

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DEFECTS OF LIVER GLYCOGEN METABOLISM IN PATIENTS WITH AGENESIS OF THE DORSAL PANCREAS

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CONTROL OF MUSCLE GLYCOGEN SYNTHASE THROUGH GLYCOGEN PHOSPHORYLASE AND PROTEIN TARGETING TO GLYCOGEN

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Background and Aims: Muscle glycogen synthesis is activated by dephosphorylation-activation of glycogen synthase (GS), which is mostly attributed to protein phosphatase 1 (PP1). PP1 activity is regulated by target proteins, such as protein targeting to glycogen (PTG), that determine its location and affinity for substrates. PTG directly binds substrate enzymes for PP1, namely GS and glycogen phosphorylase (GP), independently of glycogen. This binding is presumably regulated but little is known of its mutual interaction and operational capacity *in vivo*. We have previously shown that overexpression of PTG in skeletal muscle cells clearly modified the GS activation state, whereas GP was only slightly inactivated. Here, we have further examined the interaction between PTG and GP *in vivo* by co-expression of these two proteins. **Materials and Methods:** Studies have been performed in human muscle cultured cells transduced with adenoviruses. **Results:** Co-overexpression of GP and PTG caused minor changes in GP activation in glycogen-repleted and -depleted cells, reinforcing the notion that PTG does not promote PP1 action on GP in muscle cells. In contrast, delivery of PTG and GP caused a marked activation of GS, above that attained in cells overexpressing either PTG or GP alone. The effect on GS was more apparent in glycogen-depleted cells and was correlated to a downward shift in the electrophoretic mobility of GS, indicating dephosphorylation of the enzyme. **Conclusions:** GP enhances PTG-mediated activation of GS, although GP itself is not dephosphorylated. Our data suggests that interaction of GP with PTG constitutes a stimulatory signal of glycogen synthesis, which is maximally operative at low glycogen content. This mechanism may contribute to enhancing glycogen resynthesis in muscle.

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CHARACTERISATION OF ISOFGOMINE'S INHIBITORY EFFECT ON GLUCOSE PHOSPHORYLASE AND GLYCOGENOLYSIS IN PRIMARY RAT HEPATOCYTES.

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Background and Aims: Hepatic glucose production is significantly increased in patients with type II diabetes and is therefore an important target for lowering blood glucose. Inhibition of liver glycogen phosphorylase (GP) is one approach to lower the increased hepatic glucose production. The effect of Isofagomine, a potent GP inhibitor, has been characterized *in vitro*. **Materials and Methods:** Glycogen phosphorylase (GPa: the phosphorylated form; GPb: the unphosphorylated form) activity was assayed in the direction of glycogenolysis and glycogen synthesis. Primary hepatocytes were isolated from male Sprague Dawley rats and cultured. Glycogen synthesis was induced with insulin and glucose and subsequently basal and glucagon-stimulated glycogenolysis was measured. **Results:** When analyzed in the direction of glycogenolysis Isofagomine was found to be a potent inhibitor of rat and pig liver GPa with an IC₅₀ of 697 ± 85 nM and 773 ± 9 nM, respectively. Also, AMP-stimulated rabbit muscle GPb was inhibited with an IC₅₀ of 1.21 ± 0.18 μM. Remarkably, the IC₅₀ value was increased to 279 ± 37 μM when pig liver GPa was analyzed in the direction of glycogen synthesis. The mode of inhibition was found to be non-competitive with respect to glucose and caffeine. Likewise, the inhibitory effect of Isofagomine was not potentiated in the presence of either glucose or caffeine; glucose antagonized the effect of Isofagomine. In primary cultured rat hepatocytes Isofagomine inhibited basal and glucagon-induced glycogenolysis dose-dependently with an IC₅₀ of 3.0 ± 0.4 μM and 2.0 ± 0.5 μM, respectively. The effect of Isofagomine on glycogenolysis was observed with respect glucose production, lactate formation, and glycogen levels. Isofagomine had no effect on the enzymes involved in the covalent modification of GP: phosphorylase kinase and protein phosphatase 1. Moreover, Isofagomine had no effect on phosphoglucosylase, glucose-6-phosphatase, while the debranching enzyme was inhibited 10 % at 200 μM of Isofagomine. **Conclusions:** Isofagomine is a potent and selective inhibitor of GP and glycogenolysis in hepatocytes. Inhibition of glycogenolysis is a potential means whereby the overproduction of glucose by the liver in the diabetic state may be reversed.

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Pharmacodynamic profile of the glycogen phosphorylase inhibitor 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) in glucagon-challenged rats, rabbits and dogs.
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Background and Aims: It has previously been shown that DAB inhibits glycogenolysis in rat hepatocytes *in vitro* and reduces glycogenolysis and BG excursions in glucagon-challenged ob/ob and lean mice. Here we studied the pharmacodynamic effects of DAB in glucagon-challenged rats, rabbits and dogs.

Materials and Methods: DAB was administered at 0.04-24mg/kg i. v. to postabsorptive anaesthetised male Sprague Dawley rats just before an i. v. glucagon challenge or p. o. to conscious rats 15 min before a s. c. glucagon challenge and BG was monitored to identify ED₁₀₀ values. Those values were used to study the pharmacodynamic effects of DAB by each route by dosing 5-90 min (i. v.) or 15-120 min (p. o.) before a glucagon challenge. Danish Land Race female rabbits were dosed i. v. with 0.1-10mg/kg DAB 10 min before an i. v. glucagon challenge and the ED₁₀₀ value was subsequently used to assess t_{1/2}. In female Beagle dogs DAB was administered at 4mg/kg p. o. 2-5h before i. v. glucagon and the t_{1/2} was estimated from the AUC of BG.

Results: A log-linear DAB i. v. dose response relationship was observed in i. v. glucagon-challenged rats and 1-2mg/kg completely suppressed the 3-4mmol/l BG excursion seen with glucagon alone. The BG lowering effect of a DAB dose of 1.6mg/kg i. v. had an estimated t_{1/2} of 50 min. When dosed p. o. to rats a log-linear dose response was observed, again with 1-2mg/kg DAB completely suppressing the BG excursion. A p. o. dose of 1.2mg/kg DAB had an estimated t_{1/2} of 60 min. These data suggest very high oral availability of DAB in rats. In rabbits 3mg/kg DAB completely suppressed the 3mmol/l glucagon-induced BG excursion. The estimated t_{1/2} of this dose was 5 h. When dogs were dosed p. o. with 4mg/kg of DAB 2 or 3 h before glucagon the 3.5mmol/l BG excursion was nearly completely suppressed, with lesser effect when dosed 4 or 5 h before. T_{1/2} was estimated to 4h from the AUC.

Conclusions: These data suggest that inhibition of hepatic glycogen phosphorylase may constitute a novel therapeutic principle in type 2 diabetes patients where elevated plasma glucagon reportedly contributes to excessive hepatic glucose output.

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INHIBITION OF GLYCOGENOLYSIS HAS NO EFFECT ON GLUCONEOGENESIS AND GLYCOGEN SYNTHESIS IN FASTED RATS
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Background and Aims: Suppression of glycogen breakdown may take place via allosteric inhibition or by dephosphorylation of glycogen phosphorylase a. The latter results in activation of glycogen synthase dependent on the maintenance of glucose 6-phosphate levels. 1,4-dideoxy-1,4-imino-D-arabinitol (DAB), which represents a novel class of potent glycogen phosphorylase inhibitors, was previously shown to inhibit glycogenolysis *in vivo* without a compensatory increase of gluconeogenesis. This may reflect that gluconeogenic glucose was stored as glycogen. We therefore investigated the effects of DAB on glucose production and glycogen synthesis from gluconeogenic substrates in primary rat hepatocytes and in 24-h fasted rats *in vivo*. **Materials and Methods:** Hepatocytes were incubated in the presence of 15 mM 1-¹³C-glucose and 10 nM insulin for 24 h. Subsequently the cells were incubated in a new medium containing 3 mM 2-¹³C-glycerol, no glucose +/- 2 nM glucagon. In rats ¹³C-enriched lactate (30%) was infused to obtain a plasma level of 5 mM together with somatostatin and replacement levels of insulin. ¹³C-filtered ¹H NMR was applied to identify and quantify the position of ¹³C-enrichment in glucose and lactate. **Results:** The experimental set up in hepatocytes allows estimation of both glycogenolysis and gluconeogenesis. The data demonstrated that 25 μM DAB efficiently inhibits glycogenolysis by 70% whereas gluconeogenesis appears to be virtually unaffected. *In vivo* we observed no difference in ¹³C content of glucose (p = n.s., n = 5) with (1.41 ± 0.11 %) or without (1.47 ± 0.03 %) DAB. Further, we observed no differences in total hepatic glycogen levels with or without DAB in fasted rats. **Conclusion:** DAB inhibited glycogenolysis with no effect on glucose production or glycogen synthesis from glycerol and lactate *in vitro* and *in vivo*. These results suggest that inhibition of glycogenolysis could be an effective anti-diabetic treatment.

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DIURNAL CHANGE IN MUSCLE GLYCOGEN IN TYPE 2 DIABETES AND IN NORMAL SUBJECTS

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Background and aims: To determine diurnal change in tissue glycogen stores in relation to insulin sensitivity, subjects with type 2 diabetes of over two years duration and age and weight matched controls have been studied metabolically and by magnetic resonance spectroscopy. **Methods:** After breakfast and lunch gastrocnemius muscle glycogen was measured 2 hourly by magnetic resonance spectroscopy at 3.0 Tesla. On a separate day, a short insulin sensitivity test (0.1 units/kg) was carried out with fasting calorimetry basally and repeated 20 min after insulin injection. **Results:** After breakfast and lunch muscle glycogen increased above basal concentration by 30.2 and 54.3% respectively in normal subjects but by only 12.4 and 37.9% respectively in diabetic subjects. In a separate group of young healthy volunteers muscle glycogen increased above basal concentration by 37.1 and 99.0% respectively. This subnormal muscle storage of glucose as glycogen in type 2 diabetes was related to insulin sensitivity. Mean K_{ITT} in the diabetic group was less than 50% of that of the controls (0.93 ± 0.41mmol/l/min and 1.97 ± 0.58 mmol/l/min; p<0.01). Glucose oxidation increased from 0.97 ± 0.47 to 2.02 ± 0.74 g/min in the diabetic patients, and from 1.26 ± 0.52 to 2.83 ± 0.36 g/min in controls. **Conclusions:** The extent of slow and incomplete storage of meal-derived glucose as muscle glycogen in type 2 diabetes and its relationship to *in vivo* insulin sensitivity has been shown for the first time.

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Muscle glycogen accumulation correlates with exercise activity irrespective of dietary fat content

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Background and Aims: Impaired muscle glucose uptake and a reduced ability to store glucose as glycogen are central to insulin resistance. Exercise improves insulin sensitivity and increases insulin stimulated glucose uptake and glycogen synthase activity. High fat feeding has the opposite effect. This study investigated the effect of the amount of exercise on glycogen accumulation in quadriceps muscles of rats fed either a high fat or normal diet. **Materials and Methods:** 16 male wistar rats were fed a standard chow diet with (TC) or without (SC) access to running wheels, 16 others were fed a high fat diet (59% fat) with (TF) or without (SF) access to running wheels. An oral glucose tolerance test (OGTT) (3g glucose/kg body weight) was performed on day 25 and plasma glucose area under the curve response (GAUC, mM.min) assessed. Three days later quadriceps muscle was removed for assessment of glucose 6-phosphate (G6P) and glycogen content, active (0.1mM G6P) and total (10mM G6P) glycogen synthase (GS) activity, GS fractional velocity and glycogen phosphorylase a (GP_a) activity. **Results:** Distance travelled was not different between the trained groups. Fat feeding increased the GAUC (SC 1112 ± 28 vs SF 1231 ± 31 p = 0.02) and training decreased the GAUC (SC vs TC 970 ± 43 p = 0.02, SF vs TF 1082 ± 33, p = 0.01). Training significantly increased the quadriceps glycogen content (μmols / g dry weight) of both groups (SC 105 ± 13 vs TC 143 ± 9 p = 0.03, SF 78 ± 6 vs TF 107 ± 6 p = 0.003). The quadriceps glycogen content in trained rats was significantly positively correlated to the distance run over the 28 day period (TC R = 0.82, p = 0.01, TF R = 0.73, p = 0.04) in both chow and fat fed rats although fat feeding produced less glycogen accumulation (p = 0.003). G6P content, total GS and GP_a activities were similar in all 4 groups. GS fractional velocity decreased in both groups, (SC 0.65 ± 0.1 vs TC 0.39 ± 0.1 p = 0.02). Neither the GAUC nor enzyme activities correlated with the distance run. **Conclusions:** Quadriceps glycogen content increased proportionally to the distance traveled, irrespective of the diet. These increases in glycogen cannot be simply explained by alterations in GP_a GS or G6P. Since previous studies have shown increased insulin stimulated glycogen synthase activity after training, insulin regulation of glucose uptake and glycogen synthase must be critical to the accumulation of glycogen in trained animals.

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Insulin Interaction with prior exercise in promoting glycogen synthesis in different muscles and liver: implications for exercise-mediated changes in insulin sensitivity.

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Background and Aims: Exercise is thought to improve insulin sensitivity. Following acute exercise, this observation has not been consistent. To determine whether this might be due to a summation of non-uniform effects in different tissues, glycogen synthesis in response to insulin was evaluated in different muscles and liver in rats, following a 4h swim. **Materials and Methods:** Four groups of Sprague-Dawley rats (200-230g) underwent: (i) a 4h swim followed by a 3h recovery, SR; (ii) a 4h swim followed by a 3h insulin infusion (29pmol/kg-min), SI; (iii) 4h rest + 3h 'recovery', RR; (iv) 4h rest + 3h insulin infusion, RI. Glucose was clamped at basal during insulin infusions and [6-3H]glucose was infused during the study. Plasma tracer and glucose were measured during the 3h recovery or insulin infusion. At termination, the rats were sacrificed and soleus (S), white (WG) and red (RG) gastrocnemii, and liver were freeze-clamped. Total and labeled glycogen were determined. New synthesis was calculated from [3H] glucosyl content of glycogen divided by glucose specific activity during recovery (+/- insulin). Insulin sensitivity was estimated from the ratio of glucose metabolic clearance (MCR) and insulin levels. Statistical analysis was performed using one-way analysis of variance. **Results:** After exercise, insulin was similar: 511±177 pM for RI vs 567±105 for SI (p>0.1) but MCR increased from 26±4 to 41±4 ml/kg-min (p<0.05), indicating an increase (~50%) in insulin sensitivity (p=0.03). Total glycogen rose or remained unchanged after exercise (relative to RR) in all muscle groups, except in SR where it decreased. New glycogen synthesis (mg/g tissue) in liver, soleus, RG and WG was found to be as follows: RR: 0.1±0.1, 0.9±0.2, 0.3±0.1 and 0.1±0.1; RI: 0.5±0.2, 3.0±0.4, 3.0±0.8, 0.4±0.2; SR: 0.4±0.2, 0.7±0.3, 1.2±0.4** and 0.9±0.2**; SI: 1.9±0.4, 3.0±0.4, 8.8±0.8 and 5.1±0.2. To determine any synergistic effects of insulin, SI was also compared to the additive effects of prior swimming and insulin (SR+RI-RR): 0.8±0.2*, 2.8±0.4, 3.9±0.8*, 1.2±0.2* (* p<0.05, SI vs SR+RI-RR, ** p<0.05, SR vs RR). **Conclusions:** Insulin-stimulated new glycogen synthesis was enhanced synergistically (2-3 fold that expected from additive effects alone) by prior exercise in those muscle groups (WG and RG) where exercise alone increased glycogen synthesis. The effect was present but smaller in liver. Thus the differential nature of the effect of exercise on insulin action on glycogen synthesis in different tissues, likely attenuates the overall change in systemic insulin sensitivity.

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FENOFIBRATE DIRECTLY INHIBITS GLYCOGEN SYNTHESIS OF ISOLATED RAT SKELETAL MUSCLE.

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Aims: Fibrates are used for the treatment of lipid disorders and have been demonstrated to carry an insulin-sensitizing potential. This study was to investigate the direct effects of fibrates on skeletal muscle glucose metabolism. **Materials and Methods:** Soleus muscle strips from Sprague-Dawley rats were incubated for 90 min or 24 h in the presence or absence of fibrates. During the last hour, rates of glucose metabolism were determined in the presence of insulin. Glycogen content was measured after the experiment. **Results:** 90 min exposure of muscle specimens to fenofibrate dose-dependently inhibited insulin-stimulated glycogenesis, which resulted in decreased glycogen content (% of an intraindividual control incubated in the absence of fenofibrate: *net glycogen synthesis*: 10μM, -12±5%, p<0.05; 25μM, -16±6%, p<0.05; 50μM, -13±5%, p<0.02; 100μM, -26±4%, p<0.001; 200μM, -18±5%, p<0.001; *glycogen content*: 10μM, -4±3%, ns; 25μM, -13±4%, p<0.02; 50μM, -7±3%, p<0.05; 100μM, -13±3%, p<0.001; 200μM, -10±4%, p<0.02). Inhibition of glycogen synthesis by fenofibrate persisted after prolonged exposure for 24 h (*net glycogen synthesis*: 10μM, -7±14%, ns; 25μM, -9±15%, ns; 50μM, -20±8%, p<0.05; 100μM, -17±7%, p<0.05; 200μM, -27±7%, p<0.005; *glycogen content*: 10μM, -8±9%, ns; 25μM, -9±10%, ns; 50μM, -22±5%, p<0.001; 100μM, -20±5%, p<0.001; 200μM, -28±4%, p<0.001). Glucose oxidation, anaerobic glycolysis, and 2-deoxy-glucose transport were determined in parallel, but remained unchanged, and other fibrates failed to exhibit glycogenolytic action (exposure to 100μM of the respective fibrate for 90 min: *net glycogen synthesis*: clofibrate, -7±14%, ns; ciprofibrate, -9±15%, ns; bezafibrate, +13±21%, ns; *glycogen content*: clofibrate, -2±7%, ns; ciprofibrate, -6±5%, ns; bezafibrate, -3±14%, ns). **Conclusions:** Fenofibrate directly inhibits glycogenesis in skeletal muscle *in vitro*. Failure of other fibrates suggests a fenofibrate-specific mechanism of action, and, hence, the involvement of a pathway different from that addressed by all fibrates to improve lipid metabolism.

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QUANTITATION OF GLYCOGEN PHOSPHORYLASE ISOFORMS EXPRESSION IN NORMAL AND INSULIN RESISTANT RAT TISSUES

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Aims: Glycogen phosphorylase (GP) is a key enzyme in the regulation of hepatic glucose output. This work aimed to determine the level and pattern of expression of the liver, skeletal muscle and brain isoforms of glycogen phosphorylase within normal AP Wistar and insulin resistant Zucker rat tissues. **Materials and Methods:** Isoform specific quantitative RTPCR was carried out on cDNA synthesised from total tissue RNA using a Lightcycler machine (Roche). Tissues studied were liver, skeletal muscle, brain, heart, kidney and lung. **Results:** The major isoforms expressed in liver, skeletal muscle and brain tissues were liver (76% AP/57 %Zucker), skeletal muscle (73%AP/50%Zucker) and brain (62%AP/51%Zucker) respectively. The isoforms were differentially, but also simultaneously expressed in all tissues studied. There was no overt difference in the pattern or level of expression of any of the isoforms between normal and Zucker rat. **Conclusions:** The insulin resistance and impaired glucose tolerance seen in the Zucker fa/fa rat is not associated with any alteration in GP isoform mRNA expression levels. The presence of multiple enzyme isoforms in the tissues studied would suggest attempts to reduce hepatic glucose output by specific inhibition of the liver GP isoform could result both in additional effects on the isoform expressed elsewhere and sub-maximal inhibition of hepatic GP activity due to compensatory action of the non-targeted isoforms.

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IN VIVO AND IN VITRO EFFECTS OF THE HEPATIC GLUCOKINASE GENE PROMOTER -258 A VARIANT ON PROMOTER ACTIVITY AND INSULIN SENSITIVITY- CONTROVERSY REVISITED

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Background and Aims: The -258 G-A variant of the glucokinase (GCK) gene hepatic promoter has been reported to be associated with impaired promoter activity in vitro and insulin resistance in vivo in a small cohort of African-Americans with normal glucose tolerance (NGT). In this study, we have attempted to resolve the controversy of non-reproducible in vivo finding in other ethnic populations. **Materials and Methods:** We studied 318 unrelated Chinese subjects (aged 50.7±12.0 years; mean±SD) with NGT (M/F=43%/57%) according to WHO (1985) diagnostic criteria. Fasting and 2-hour plasma glucose and insulin levels were measured during a 75-g oral glucose tolerance test. The Homeostasis Model Assessment of insulin resistance (HOMA-IR), was calculated. Genotype frequency was determined by AclI restriction fragment length polymorphism. PGL3 vector (with a luciferase report gene containing a 0.7 kb (-1 to 700 including the mutant site) or a 1.7 kb fragment (including an upstream liver specific enhancer -1000 to -700) of glucokinase promoter was transfected into HepG2 cells along with PGL3-GCK promoter clones and pCMVb-gal internal control plasmid. The wild-type (WT) and mutant clones were confirmed by sequencing. Cells were harvested in lysis buffer containing protease inhibitors after 72-hour transfection. The luciferase and b-gal activities were assayed using Promega assay kits. **Results:** The mean promoter activity was 3058 U* (100%) in the (WT) (GG) and 1788 U (58%) in the mutant (AA) for the 0.7kb fragment (n=3); 6934 U (100%) in the WT (GG) and 6958 U (100%) in the mutant (AA) for the 1.7kb fragment (n=2). Fasting and 2-hour insulin levels and HOMA-IR were not significantly different between subjects with the A allele (allele frequency 0.24) (5.50 ±0.32 vs 5.36±0.23 mU/L for GG, 51.20±5.61 vs 53.49±3.82 mU/L for GG, 1.25±0.07 vs 1.21 ±0.05 for GG respectively; mean±SEM) and those with the GG genotype. **Conclusions:** These data suggest that the -258 hepatic glucokinase gene promoter variant is not associated with insulin resistance in Chinese subjects with normal glucose tolerance, similar to finding in Danish Caucasians. In the hepatic glucokinase gene, while a short fragment of promoter containing this variant has decreased promoter activity, any upstream liver specific enhancer in the promoter can lead to the negation of this effect resulting in no observable reduction in insulin sensitivity in vivo.

* arbitrary unit after adjustment for b-galactosidase activity and amount of protein

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REGULATION OF BLOOD GLUCOSE METABOLISM IN MICE EXPRESSING HIGH AND LOW HEPATIC GLUCOKINASE ACTIVITY

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Background and Aims: Glucokinase (GLK) is a key regulatory enzyme in hepatic glucose storage and pancreatic insulin secretion and enzyme mutations can cause a form of MODY. Strains of mice have a two-fold difference in hepatic glucokinase activity, with C3H being high and C58 is low. Oral fructose administration to mice will lower blood glucose levels immediately after an oral glucose load (possibly via release of GLK from its regulatory protein (GLKRP) through formation of fructose-1-phosphate). We determined the effect of an oral fructose administration to C3H and C58 mice on plasma glucose levels and related these results to levels of GLKRP levels in the liver. Both strains of mice were given, orally, glucose (2 g/kg) or glucose and fructose (both at 2 g/kg). **Results:** The plasma glucose levels after glucose administration were 20.83 ± 0.97 versus 18.32 ± 1.00 mM (P<0.09) in C3H and C58 mice respectively. Fructose significantly lowered the glucose levels in the C3H mice from 20.83 ± 0.97 to 17.22 ± 1.03 mM (P<0.009) but did not significantly lower blood glucose levels C58 mice (18.32 ± 1.00 versus 16.41 ± 0.78 (P<0.075)). Plasma lactate levels were significantly higher in C3H mice versus C58 mice (6.20 ± 0.60 versus 4.02 ± 0.34 (P<0.005)). Administration of fructose did not affect plasma lactate levels in either strain of mice.

Conclusions: Mice (C3H) with higher levels of liver glucokinase activity were able to decrease elevated blood glucose levels when given fructose orally. C3H mice have also higher expression levels of GLKRP than C58 mice which suggests that the level of expression GLK and GLKRP may play a role in hepatic glucose handling.

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Lipid Metabolism

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Do postprandial lipoproteins regulate adhesion molecules ?

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Background and Aims: Type 2 diabetes is associated with up to 5-fold increase in atherosclerosis. Adhesion molecules are a marker for atherosclerosis. Regulation of gene expression by fatty acids depends on fatty acid structure, and we have previously demonstrated major alterations in the postprandial lipoproteins in diabetic patients particularly on a linoleic acid diet. The purpose of this study was to explore the relationship between postprandial lipoproteins and adhesion molecules on different fatty acid diets. **Materials and Methods:** Nine type 2 diabetic and 9 non-diabetic subjects (HbA1c 7.0±1.0%) were examined 4 weeks after the start of a diet enriched with either oleic or linoleic acid in a randomised cross-over study. Fasting and postprandial blood samples were taken following an 1100 kcal fat-rich meal at the end of each dietary period. Vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) and e-selectin were measured by Elisa. Apo B48 and apo B100 were measured by gradient gel electrophoresis following ultracentrifugation. **Results:** Adhesion molecules were not significantly different between diabetic and control subjects. For the diabetic patients ICAM on the linoleic acid diet was 317±44 ng/ml plasma vs 290±35 ng/ml plasma on oleic acid. VCAM was 388±73 on linoleic acid vs 403±123 ng/ml on oleic acid and e-selectin was 49±20 on linoleic acid vs 54±28 ng/ml on oleic acid. There was a significant increase in chylomicron apo B48 (p<0.05) and therefore of chylomicron number, in diabetic patients on the linoleic acid diet. There was no correlation between HbA1c or blood sugar and any of the adhesion molecules. There was a significant positive correlation between postprandial chylomicron apo B48 and e-selectin on linoleic acid (r=0.47, p<0.05) and oleic acid (r=0.49, p<0.05) diets and also between postprandial chylomicron apo B100 and e-selectin on linoleic acid (r=0.45, p<0.05) and oleic acid (r=0.5, p<0.05). Chylomicron apo B48 was significantly related to ICAM on oleic acid diet (r=0.68, p<0.001). There was no significant correlation between any of the postprandial lipid components of the chylomicron and the adhesion molecules measured. **Conclusions:** This study suggests that adhesion molecules may in part be regulated by postprandial lipoproteins and that the fatty acid composition of the diet may influence this regulation.

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Title: STIMULATION OF HUMAN MUSCLE GLYCOGEN PHOSPHORYLASE GENE EXPRESSION BY UNSATURATED FATTY ACIDS.

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Background and aims: Muscle glycogen phosphorylase (MGP) expression is elevated in obesity-associated human type 2 diabetes. Hormones, such as epinephrine or insulin, have been suggested to participate in the induction of the gene. In this study, we examined the possibility that increased MGP gene expression was due to regulation by unsaturated fatty acids and the mechanism of such effect. **Methods:** Muscle cultures were established by growth and differentiation of myoblasts obtained from human biopsies; total MGP activity was determined in total cell extracts in the presence of the activator AMP; [U-¹⁴C]-glycogen was isolated from total cell extracts by precipitation in cold 66% ethanol. **Results:** When human skeletal muscle cells were incubated for 16 h with 0.5 mM oleate sodium salt, total MGP activity was increased from 46 ±3 to 69 ±5 mU/mg protein, whereas treatment with palmitate had no effect. Consistently, [U-¹⁴C]-glucose incorporation into glycogen decreased in oleate-treated cells. Sequence analysis of the human MGP promoter revealed the presence of a putative peroxisomal proliferator response element (PPRE) that could be involved in such effect. Thus, the modulation of MGP activity levels in response to peroxisome proliferator-activated receptor (PPAR) ligands was studied. Incubation of cells with a selective PPARγ ligand increased MGP activity, whereas a PPARα ligand showed no effect. A MGP promoter-GFP reporter construct was delivered to muscle cells by means of adenovirus. The promoter activity was increased after treatment with oleate, linoleate or PPAR agonists. **Conclusions:** Our data shows that MGP gene expression is stimulated by unsaturated fatty acids. The presence of a PPRE in the MGP promoter and its sensitivity to PPARs suggests that the effect of unsaturated fatty acids involves PPAR-mediated transcriptional effects.

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Altered postprandial responses in type 2 diabetes

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Background and Aims: People with type 2 diabetes are at increased risk of coronary heart disease (CHD), which may in part be due to altered postprandial metabolic responses. Alterations in lipoprotein clearance have been demonstrated in diabetic patients with fasting hypertriglyceridaemia, however a standardised method of examining postprandial lipaemia is not available. We have developed an Oral Triglyceride Tolerance Test (OTTT) which has been used to investigate post challenge metabolic changes in patients with stable type 2 diabetes. **Materials and Methods:** A 200 ml test drink containing 50g long chain triglyceride emulsion and 50g maltodextrin was administered to 30 type 2 diabetic subjects treated with diet alone (n=10), sulphonylurea (n=10), or metformin (n=10) and 20 non-diabetic subjects. Plasma lipid profiles, non-esterified fatty acid (NEFA), glycerol, glucose and insulin levels were measured fasting and post challenge two hourly for eight hours. **Results:** The diabetic and non-diabetic subjects were, respectively, mean (SD) age 55.5 (7.4) and 52.1 (9.0) years, BMI 32.7 (6.2) and 27.9 (5.6) kg/m², geometric mean (1SD range) triglyceride 1.47 (0.91 to 2.4) and 0.80 (0.52 to 1.25) mmol/L with, in the diabetic subjects, mean HbA1c 7.6 (1.2) % and fasting plasma glucose 9.1 (2.7) mmol/L. Diabetic subjects exhibited significantly higher geometric mean triglyceride levels at all time points compared to non-diabetic subjects, the greatest difference being at six hours (1.96 (1.2 to 3.3) vs 1.05 (0.6 to 1.8) mmol/L, p=0.0002). Six hour triglyceride correlated strongly with fasting triglyceride (r=0.89, p<0.0001) and incremental triglyceride AUC correlated with fasting plasma glucose (r=0.28, p=0.043). Diabetic subjects had higher mean glycerol at six hours (13.4 (5.5) vs 9.3 (2.0) mg/dL, p<0.002) and higher incremental mean AUC NEFA (p<0.01). Post challenge geometric mean insulin levels were highest (peak 300.3 (184.5 to 488.7) vs 192.4 (90.4 to 409.5) pmol/L, p=0.025) at two hours and more prolonged in diabetic subjects, but returned to baseline by eight hours. **Conclusions:** Exaggerated post challenge triglyceride, glycerol, NEFA and insulin changes are evident even in patients with moderately well-controlled type 2 diabetes. Metabolic abnormalities in the postprandial state may contribute, through accelerated atherogenesis, to the increased risk of CHD seen in these individuals.

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ROSUVASTATIN ALONE AND IN COMBINATION WITH FENOFIBRATE IN HYPERLIPIDAEMIC PATIENTS WITH TYPE 2 DIABETESP. Durrington¹, A. Hamann², J. Tuomilehto³, K. Smith⁴ and D. Kallend⁴. ¹University of Manchester, Manchester, UK; ²Universität Heidelberg, Heidelberg, Germany; ³National Public Health Institute, Helsinki, Finland; ⁴AstraZeneca, Alderley Park, UK.

Background & Aims: This double-blind, multinational, multicentre study (4522IL0036) compared the efficacy of rosuvastatin (ROS; Crestor™) in reducing triglyceride (TG) levels with that of fenofibrate (FEN) and 2 ROS+FEN combinations in Type 2 diabetic patients with hypertriglyceridaemia. **Materials & Methods:** After a 6-wk dietary lead-in, 216 diabetic men and women (>18yrs; fasting TG ≥2.26 but <9.03mmol/L; total cholesterol (TC) ≥5.17mmol/L; HbA1c <10%) were randomised to ROS 5 or 10mg, or 1 of 2 placebo (PLA) arms for 6wks. Patients with low-density lipoprotein cholesterol (LDL-C) ≥1.3 mmol/L were then force-titrated in an 18-wk, open-label phase as follows: PLA group 1 received ROS (three 6-wk periods at 10, 20, then 40mg/d); PLA group 2 received FEN 67mg (od, bd, then tds); and ROS 5- and 10-mg groups continued to receive the same dose plus force-titrated FEN 67mg. Pairwise t-tests (ANOVA) were used to compare force-titrated ROS with other treatment regimens. **Results:** At wk 6, ROS 5 and 10mg significantly reduced TG, LDL-C, TC, and Apo B and increased high-density lipoprotein cholesterol (HDL-C) over combined PLA group (p<0.001). Lipid results at wk 24 of the force-titration phase are shown below (Table). All treatments, including ROS+FEN combinations, were well tolerated over 24wks. **Conclusions:** The ROS 10mg+FEN combination yielded a greater reduction in TG than those seen with the other treatments, with a significantly greater reduction over force-titrated ROS. Force-titrated ROS and FEN were similarly effective in reducing TG levels. Both ROS 5 and 10mg improved the atherogenic lipid profile in Type 2 diabetic patients.

Lipid Parameter	ROS 40mg od (n=51)	FEN 67mg tds (n=49)	ROS 5mg od+ FEN 67mg tds (n=60)	ROS 10mg od+ FEN 67mg tds (n=53)
TG BL (SD)	3.6 (1.0)	4.2 (1.8)	3.5 (1.2)	3.5 (1.3)
WG 24 %Δ (SE)	-30 (4)	-34 (4)	-41 (4)	-47* (4)
LDL-C BL (SD)	3.7 (0.7)	3.7 (0.8)	3.9 (0.8)	3.9 (0.8)
WG 24 %Δ (SE)	-47 (3)	1* (3)	-34* (3)	-42 (3)
TC BL (SD)	6.2 (0.7)	6.3 (0.9)	6.5 (0.8)	6.4 (0.9)
WG 24 %Δ (SE)	-37 (2)	-7* (2)	-31 (2)	-36 (2)
HDL-C BL (SD)	1.0 (0.2)	1.0 (0.2)	1.1 (0.2)	1.0 (0.2)
WG 24 %Δ (SE)	6 (2)	9 (2)	11 (2)	12 (2)

*p<0.017 vs ROS 40mg od [NB: Prespecified level of statistical significance controlled for multiple comparisons]; BL = baseline [NB: Baseline values in mmol/L]; %Δ = percentage change from baseline

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HYPERLIPIDEMIA AND GLUCOSE LEVEL CONTROL IN TYPE 2 DIABETES MELLITUS PATIENTS FROM WEST OF MEXICO.

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Background: The most common pattern of dyslipidemia in type 2 DM is increased triacylglyceride levels and decreased HDL cholesterol. Also it is believed that control of glucose levels may improve the dyslipidemic stage. The aim of this study was to determine the different hyperlipidemias present in patients with type 2 DM from the west of Mexico and analyze the lipid profile associated to serum glucose levels. **Materials and methods:** One hundred six patients (55.3±10.4 years old) with type 2 DM and 436 healthy volunteers (HV) (45.92 ±16 years old) were studied. Serum lipid profile, glucose and HbA1c were analyzed using conventional biochemical methods. Patients were classified in groups according fasting serum glucose levels: 1) <140 mg/dl; 2) 141-180 and 3) > 180 mg/dl. The hyperlipidemia was analyzed according to Fredrickson classification. **Results:** Eighty three percent of patients with type 2 DM presented dyslipidemia vs 39.4% in HV group (p<0.000001). Twenty six percent of the HV group presented type II hyperlipidemia, whereas in diabetic patients hyperlipidemia type III and IV were more common with 28% and 23% respectively. The frequency of hyperlipidemia in patients with type 2 DM was no different between groups according to glucose levels. A negative relationship was found between glucose levels and apo A-I (correlation coefficient = -0.59, p<0.01). **Conclusions:** Control of glucose levels not necessarily improves the dyslipidemia, suggesting that specific measures have to be taken for dyslipidemia treatment in type 2 DM patients. This study also suggest that lipid glucose level facilitate HDL uptake by the liver, decreasing apo A-I levels and increasing the risk for cardiovascular disease.

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INSULIN FAILS TO NORMALIZE FREE FATTY ACID CLEARANCE IN TYPE 2 DIABETES: A POSSIBLE FACTOR FOR PREMATURE ATHEROSCLEROSIS

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Background and Aims: Premature atherosclerosis frequently occurs in type 2 diabetes (T2DM) despite near optimal glycemic control. This has been partially attributed to persistently impaired metabolism of triglyceride rich lipoproteins (TRL) due to elevated plasma free fatty acids (FFA). Increased fasting plasma FFA in T2DM are due to reduced FFA clearance (C) since FFA release has uniformly been shown to be normal. We therefore assessed whether acute insulin infusion, which produces normoglycemia, would also normalize FFA C in T2DM. **Materials and Methods:** We determined plasma concentrations, systemic turnover (T) and C of glucose and FFA using tracer techniques (6-³H glucose and 9,10-³H palmitate) in 28 subjects with T2DM with (DM+, n = 10) and without an algorithm based variable overnight insulin infusion (DM-, n = 18) to restore fasting normoglycemia as well as in 24 matched nondiabetic controls (NC). **Results:** Overnight insulin normalized plasma glucose in DM+ (5.3 ± 0.1 vs 9.3 ± 0.6 in DM- and 5.2 ± 0.1 mM in NC) and resulted in ~2-fold greater plasma insulin levels (~135 compared to DM- and NC (~60 pM). Glucose T and C were normalized by insulin in DM+ (10.1 ± 0.7 and 1.93 ± 0.16 vs 14.6 ± 0.8 and 1.63 ± 0.08 in DM-, both p < 0.01 and vs 10.6 ± 0.4 μmol/kg/min and 2.05 ± 0.06 mL/kg/min in NC, both p > 0.7). Plasma FFA decreased in DM+ (450 ± 45 vs 613 ± 33 μM in DM-, p < 0.05) and were comparable to values in NC (476 ± 42 μM, p > 0.9). In contrast, FFA C remained ~50% reduced in DM+ (7.2 ± 1.0 vs 11.4 ± 1.2 mL/kg/min in NC, p < 0.05) and was similar to DM- (7.3 ± 0.5 mL/kg/min). The reduction in plasma FFA in DM+ was due to the suppression of FFA T below normal (4.04 ± 0.45 vs 5.40 ± 0.27 in DM- and vs 5.25 ± 0.25 in NC, both p < 0.04). **Conclusions:** Infusion of insulin, which restores normoglycemia, normalizes glucose T and C but fails to normalize FFA C in T2DM. This may be an important factor for premature atherosclerosis and suggests a fundamental defect in T2DM.

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Gene Expression of Cytosolic Phosphoenolpyruvate Carboxykinase in Subcutaneous Adipose Tissue Positively Correlates with Body Mass Index and Plasma Triglyceride Level

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Background and Aims: Increased visceral adiposity is a strong risk factor for insulin resistance, diabetes, and mortality from arteriosclerotic disease. Free fatty acids (FFA) and glycerol released by increased omental fat is also a risk factor for insulin resistance syndrome. On the other hand, phosphoenolpyruvate carboxykinase (PEPCK) is the rate-limiting enzyme of gluconeogenesis in the liver and kidney and of glyceroneogenesis in white and brown adipose tissue under tissue-specific regulations. Glyceroneogenesis is required for re-esterification of FFA to maintain an active level of triglyceride synthesis even during period of net lipolysis. Based on our understanding of insulin resistance syndrome, we will test whether PEPCK gene expression in visceral fat or subcutaneous fat is different between obese patients and non-obese patients without diabetes and whether PEPCK gene expression correlates with serum level of insulin, triglyceride, fasting glucose, and the degree of insulin sensitivity. **Materials and Methods:** Twenty-two non-obese non-diabetic subjects undergoing laparotomy for the benign intra-abdominal diseases were recruited. Seventy-six obese (BMI \geq 30) non-diabetic subjects undergoing laparotomy for gastric partitioning were recruited. During laparotomy, subcutaneous and visceral fat were obtained. Then real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR) for cytosolic PEPCK gene were performed to quantify the gene expression level of PEPCK gene in the two different adipose tissues. **Results:** There are higher expression level of PEPCK gene in subcutaneous adipose tissue (obese: 4.6 ± 7.6 , non-obese: 1.46 ± 1.6), BMI (obese: 40.0 ± 6.0 , non-obese: 23.3 ± 3.6), fasting plasma sugar level (obese: 98.4 ± 12.8 , non-obese: 92.6 ± 13.1), fasting cholesterol level (obese: 199.5 ± 32.8 , non-obese: 156.7 ± 25.6), fasting triglyceride level (obese: 193.7 ± 128.2 , non-obese: 68.3 ± 24.9), fasting insulin level (obese: 21.3 ± 11.8 , non-obese: 15.4 ± 15.7), and HOMA IR index (obese: 5.1 ± 2.9 , non-obese: 3.6 ± 3.6) in obese subjects than that in non-obese subjects. PEPCK gene expression level was positively correlated with BMI ($r=0.324$, $p=0.004$) and fasting triglyceride concentration ($r=0.278$, $p=0.014$). **Conclusions:** In this study, obese subjects have higher PEPCK gene expression in subcutaneous adipose tissue, fasting plasma sugar level, fasting triglyceride level, and HOMA IR index than that in non-obese subjects. Besides, PEPCK gene expression level in subcutaneous adipose tissue was positively correlated with BMI and fasting triglyceride level. Therefore, we inferred that PEPCK gene expression in adipose tissue may play a role on the pathogenesis of insulin resistance.

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Insulin Resistance and Postprandial Lipoprotein Remnant Particle Accumulation Precedes other metabolic and Body Compositional Changes in the Pre-Diabetic men.
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Background: Several perturbations are demonstrated in first-degree relatives of type 2 diabetes. Correctly defining the primary defect may improve the means of intervention in order to prevent type 2 diabetes and its sequelae.

Material and Methods: In this cross-sectional study we recruited 17 non-diabetic men with at least two first-degree relatives with type 2 diabetes. They were individually matched with 17 control subjects without known diabetes heredity for: age (48 ± 5 vs. 48 ± 7 y), body mass index (26.6 ± 2.5 vs. 26.2 ± 2.5 kg/m²) and fasting triglyceride level (1.07 ± 0.38 vs. 1.02 ± 0.31 mmol/l). The fasting glucose level at screening was normal in both groups (4.8 ± 0.3 vs. 4.7 ± 0.5 mmol/l). The investigations performed were intravenous glucose tolerance test (IVGTT), euglycemic hyperinsulinemic clamp (60 mU/m²/min), 8-h meal tolerance test (919 kcal, 51 g fat) during which lipoproteins were separated by density gradient ultracentrifugation, peak VO₂ and computerized tomography (CT).

Results: Despite rigorous matching the relatives exhibited a 30% reduction in insulin sensitivity compared to the controls (Insulin Sensitivity Index; 0.09 ± 0.01 vs. 0.13 ± 0.01 mg*ml/kgLBM*min* μ U, $p=0.031$). In contrast, the relatives had normal first-phase insulin secretion (441 ± 98 vs. 488 ± 107 μ U/ml, ns), fasting insulin level (9.6 ± 0.9 vs. 8.7 ± 0.8 μ U/ml, ns), HDL cholesterol level (1.39 ± 0.05 vs. 1.47 ± 0.09 mmol/l, ns), free fatty acids suppression during the clamp (0.12 ± 0.02 vs. 0.12 ± 0.02 mmol/l, ns) and peak VO₂ (36.2 ± 1.1 vs. 37.2 ± 1.5 ml/kg/min, ns). Moreover, abdominal fat mass was similar whether expressed as W/H-ratio (0.95 ± 0.01 vs. 0.95 ± 0.01 , ns) or visceral adipose tissue area at the lumbar 4 level (118 ± 12 vs. 127 ± 10 cm², ns). Notably, the relatives had a 30% higher level of chylomicron remnants after the meal tolerance test (VLDL1 apoB48 AUC 901 ± 117 vs. 625 ± 90 mg/l, $p=0.022$), in spite of normal triglyceride AUC. This in turn was weakly related to the degree of insulin sensitivity ($r^2=0.14$, $p=0.050$).

Conclusion: Insulin resistance and accumulation of small, atherogenic lipoprotein particles precedes typical alterations in the early course of development of type 2 diabetes in men. However, the exact nature of the relationship between these, and whether normalized insulin sensitivity will correct the lipid disturbance, remains to be established.

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Is Leptin important?

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Protein bound but not free leptin is related to sympathetic outflow in non-obese subjects.

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Background and Aims: Animal studies suggest a strong dose-dependant interaction between leptin and sympathetic nervous system activity (SNS). In humans data on a correlation of leptin levels and SNS activity are conflicting. Here we tested the hypothesis that components of the leptin system levels are related to basal sympathetic activity.

Materials and Methods: In 14 healthy subjects (10 male, 4 female, 27 ± 7 yrs, BMI: 23 ± 3 kg/m²) free and protein bound leptin was measured by specific RIA as previously described. Basal and stimulated SNS activity was determined by microneuronographic measurement of muscle sympathetic nerve activity (MSNA). In addition ECG, blood pressure, and respiration were measured continuously. Subjects underwent handgrip testing (3 min, 30 % maximum voluntary contraction), cold pressor testing (1 min), and incremental sodium nitroprusside infusions (snp; 0.2, 0.4, 0.8, and 1.6 μ g/kg/min). Total, free, and bound plasma leptin levels were measured at baseline, before and immediately after handgrip testing, 10 min after handgrip testing, and after snp infusion.

Results: The R-R interval decreased from 984 ± 125 ms at baseline to 689 ± 69 ms during snp ($P<0.001$). Blood pressure was $113\pm 7/68\pm 8$ mmHg at baseline and $104\pm 3/51\pm 3$ mmHg during snp. MSNA increased to 210 ± 77 % during snp infusion. Neither total nor free or bound leptin concentrations change during stimulation. However, MSNA (bursts/100 heart beats) was significantly correlated with bound leptin concentration ($r^2=0.48$, $P<0.05$) but not with free leptin levels ($r^2=0.00$, n.s.).

Conclusions: Our data suggest that bound but not free or total leptin levels are correlated with basal sympathetic outflow in normotensive, non-obese subjects. This fits to the close correlation demonstrated previously between bound leptin and resting energy expenditure and suggests that bound leptin may reflect basal SNS activity.

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Predictors of muscle oxygen supply in adolescents

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Background and Aims: Muscle blood flow affects insulin sensitivity in both healthy and hypertensive or insulin resistant subjects. The aims of this study were to assess the importance of muscle oxygen supply in predicting insulin sensitivity and to assess the predictive factors defining muscle oxygen supply in late adolescence.

Materials and Methods: We assessed forearm muscle of 40 healthy subjects (15-18yrs, 20 male) using near infra-red spectroscopy. Muscle reoxygenation half-time after ischaemic exercise (a nitric oxide-mediated phenomenon independent of subcutaneous fat superficial to the muscle) was related to the subjects' anthropometry, fasting plasma glucose, insulin and leptin levels, % body fat (from 5 skinfold thicknesses) and abdominal fat volume (from magnetic resonance imaging), estimates of dietary intake and physical activity (from diary records) using Pearson's correlation then stepwise regression analyses.

Results: There were no significant relationships between re-oxygenation rate and the BMI, waist circumference, abdominal visceral fat volume, physical activity or dietary intake. Significant correlations with muscle re-oxygenation half-time included: fasting insulin ($R=0.64$, $p<0.001$), fasting leptin ($R=0.93$, $p<0.001$), % body fat ($R=0.71$, $p<0.001$), abdominal sub-cutaneous fat volume ($R=0.54$, $p=0.003$). Step-wise regression analysis could not improve the association between leptin and muscle re-oxygenation rate.

Conclusions: Muscle reoxygenation rate in normotensive adolescents is related to insulin action and can be best predicted by measuring leptin levels. While leptin is reported to have differing acute effects on peripheral vasculature in vivo and in vitro, hyperleptinaemia in vivo is associated with reduced muscle perfusion.

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Low circulating free fatty-acids reduce expression of the ob-gene in white adipose tissue in post-obesity status

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Background and Aims: Recent studies indicate a tight association between obesity and high levels of leptin either mRNA or protein. Plasma leptin values in humans decrease after bariatric surgery (Bilio Pancreatic Diversion: BPD). Aim of the present paper was to investigate the regulation of the gene expression of leptin in subcutaneous adipose tissue biopsies from 14 morbidly obese women (BMI: 51.6 ± 8.2 kg/m²) before and 6 months after BPD.

Materials and Methods: Using reverse transcriptase polymerase chain reaction analysis, the mRNA expression of leptin was investigated in adipose tissue. Plasma leptin was measured by radioimmunoassay; plasma insulin by microparticle enzyme immunoassay. Free fatty acids (FFA) were measured using a colorimetric kit.

Results: A significant decrease in leptin mRNA level was observed in comparison with pre treatment in BPD patients (59 ± 34 vs 143 ± 85 R.A.; $p < 0.01$). A strict relationship between adipose tissue mRNA leptin and plasma leptin ($R^2 = 0.86$, $p < 0.0001$) and between plasma FFA concentration and insulin ($R^2 = 0.92$, $p < 0.0001$) was observed.

Conclusions: These data demonstrate a high correlation between leptin mRNA expression in adipose tissue and plasma leptin in postobese subjects after BPD. The significant relationship between leptin mRNA of adipose tissue and plasma leptin with insulin suggest that leptin expression might be regulated by circulating insulin. Finally, plasma FFA concentration seem to act as a first step on the insulin and subsequently in the leptin secretory pathway.

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Ghrelin, a gut hormone, correlates negatively to leptin in humans

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Background and aims: Ghrelin, a novel gut-brain peptide, acts in the regulation of growth hormone secretion. As it was isolated from the stomach both in rodents as well as in humans there are evidence of its participation also in energy metabolism. Ghrelin gene expression was increased by fasting and decreased by leptin. Human obesity has been considered as a leptin resistance state as serum levels are elevated in obesity matched by body mass index. The aim of this study is to verify the relationship of ghrelin and leptin in a wide variation of BMI in humans.

Material and Methods: We studied 14 severe obese patients and 14 normal controls. Ghrelin was measured by radioimmunoassay using a commercial kit provided by Phoenix. Intra-assay variation coefficient was 13.3%. Leptin was analyzed using a kit provided by Linco Co with intra-assay variation coefficient of 10.5%. We also analyzed insulin resistance by Homa model.

Results: Normal controls have BMI of 24.2 ± 1.5 , leptin levels were 7.0 ± 3.9 , ghrelin of 67.1 ± 11.9 and Homa-ir of 2.6 ± 1.0 . The severely obese patients have the BMI of 56.3 ± 10.2 , leptin of 80.2 ± 30.2 , ghrelin of 23.2 ± 6.7 and de Homa-ir of 16.2 ± 9.5 . Multivariate analysis have shown an inverse correlation of ghrelin and leptin, $r = -0.51$ ($p < 0.01$).

Conclusions: We observed in this study that ghrelin, a gut-brain peptide is maintained blunted in severely obese patients and correlated to leptin levels. We can assume that in severely obese patients, leptin, a good marker of fat tissue size can interfere to this signaling brain peptide in the central regulation of feeding.

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Ghrelin in human obesity across a range of glucose tolerance from normal to diabetes: effect of massive weight reduction

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Background and Aims: Ghrelin, an endogenous ligand for growth hormone secretagogue receptor (GHS-Rs), regulates pituitary growth hormone secretion. Peripheral administration of Ghrelin caused weight gain in mice and rats. Intracerebroventricular administration of Ghrelin generated an increase in food intake and body weight. Observing these interesting points and the possible interaction of Ghrelin in human obesity and metabolism, we performed a study concerning Ghrelin concentrations in severe human obesity ranging from normal glucose tolerance to diabetes before and after massive weight reduction.

Materials and Methods: Longitudinal clinical intervention study, in 14 severely obese women (BMI: 56.3 ± 10.2 kg/m²), classified according to glucose tolerance (8 normal - NGT group, and 6 type 2 diabetes - DM group), age: 32-55 years. Groups were matched for age and BMI. They had their levels of insulin, leptin and ghrelin evaluated by commercial RIA (Linco for insulin and leptin, and Phoenix for ghrelin) at baseline and 1-year after a bariatric surgical approach (vertical banded gastroplasty Roux-en Y gastric bypass). Insulin resistance was assessed by Homa model.

Results: At baseline, there was a significant difference in Homa-IR between groups (NGT = 14.3 ± 3.2 x DM = 20.3 ± 4.4 , $p < 0.05$), but not in insulin, leptin, and ghrelin levels. After surgery, we found a massive weight reduction similar in both groups (final BMI = 36.2 ± 7.8 kg/m², $p < 0.01$). Homa-IR had a marked reduction after surgery in NGT (3.0 ± 2.1 , $p < 0.01$), as well in DM (2.8 ± 0.8 , $p < 0.01$), being both groups with similar Homa-IR at 1-year follow-up. Insulin decreased in NGT from 44.1 ± 7.7 to 14.7 ± 3.8 uU/ml ($p < 0.01$), and in the DM group from 59.4 ± 12.8 to 15.5 ± 10.7 uU/ml ($p < 0.01$). Leptin decreased in NGT from 74.5 ± 24.9 to 20.9 ± 13.2 ng/ml ($p < 0.01$), and in the DM group from 82.9 ± 41.2 to 18.4 ± 10.1 ng/ml ($p < 0.01$). Ghrelin did not show any differences between groups and did not change after weight reduction (NGT: 21.3 ± 6.7 to 29.4 ± 4.9 pg/ml, and DM: 27.7 ± 4.9 to 24.0 ± 2.3 pg/ml). Finally, using univariate regression analysis we did not find correlation between ghrelin and other parameters.

Conclusions: Ghrelin were affected neither by glucose tolerance status, nor by weight changes in severely obese women. Despite these results, the role of ghrelin in human obesity, food intake regulation, and weight changes remains to be clarified.

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MATERNAL LEPTIN ADMINISTRATION INDUCES RESISTANCE TO DIET-INDUCED OBESITY OF EARLY GROWTH RESTRICTED RATS

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Background and aims: Epidemiological studies highlight the relationship between maternal protein restriction, early growth retardation (EGR) and an increased occurrence of adult disease. In rats, offspring of protein-deprived mothers show decreased β cell mass at birth, early onset of abdominal obesity and diabetes. We have previously demonstrated that leptin can induce proliferation of pancreatic β cells. Thus we examined the effects of leptin on the development of obesity in rats which were EGR during fetal and early post-natal life. **Materials and Methods:** Pregnant rats were fed either a control diet (20% protein) or an isocaloric diet containing 8% protein throughout pregnancy and lactation. On day 14 of pregnancy, rats (n=8) were assigned: control 20% protein group (NP), 8% protein group (LP) and 8% protein/leptin group (LPL). Each rat received a continuous infusion of saline or leptin for 28 days via a s.c. mini-pump. Pups were weaned at day 21 onto the control diet and at 6 weeks males (n=6-8) were transferred to either control or a high fat diet until 8 months of age. Results are expressed as mean (SEM). **Results:** Leptin infusion elevated plasma levels in LPL rats ($p < 0.001$) [14840(933), 3742(429), 3255(527) pg/ml: LPL, LP and NP respectively]. Leptin significantly ($p < 0.001$) reduced food intake during pregnancy [13.2(1), 22(1.3), 21(1) g: LPL, LP and NP], and body weight ($p < 0.001$) following birth [204(6), 232(7), 257(9) g: LPL, LP and NP]. Both LPL and LP pups were significantly ($p < 0.001$) smaller than NP pups at birth [5.9(0.1), 6.1(0.3), 7.3(0.1) g: LPL, LP, NP]. By 8 months, both the high fat fed NP ($p < 0.05$) and LP ($p < 0.05$), but not the LPL group, had increased body weight as compared with the chow controls [634(20)/574(6):NP, 485(10)/444(19):LP, 431(11)/432(3) g:LPL]. This was associated with increased epididymal fat pad weights [8.8(0.3), 6.5(0.4), 5(0.3) g: NP, LP and LPL] although food intake did not differ between the high fat groups. **Conclusion:** Leptin administration during pregnancy and lactation can inhibit abdominal obesity accelerated by high fat feeding of EGR rats.

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PRO-OPIMELANOCORTIN GENE HAPLOTYPE IS ASSOCIATED WITH SERUM LEPTIN LEVELS IN LEAN BUT NOT IN OBESE INDIVIDUALS

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Background and Aims: Mutations in the pro-opiomelanocortin (POMC) gene cause monogenic obesity and the POMC locus (2p21) has been linked to leptin levels and BMI. The aims of the present study were to determine frequency of monogenic obesity due to mutations in POMC or melanocortin 4 receptor (MC4R) genes among morbidly obese Swedes, and to study whether common POMC gene variants are associated with BMI or serum leptin levels among obese and lean individuals.

Materials and Methods: 102 obese individuals from Sweden (m/f 20/82, Age 40 ± 11 y, BMI 41.3 ± 5.0 kg/m²), all treated with gastric banding, were screened for mutations in the POMC and MC4R genes using SSCP and sequencing. Frequencies of the detected (POMC) variants and the POMC 5'UTR RsaI polymorphism were further studied in 118 lean control subjects (m/f 48/70, Age 56 ± 11 y, BMI 22.6 ± 1.3 kg/m²). **Results:** No cases with monogenic obesity due to mutations in POMC or MC4R genes were identified. Three common POMC gene variations were found; a 9bp insertion at codon 56 (ins56), a Glu188Gly in exon 3 and a C8246T substitution in 3'UTR. None of these variants nor the RsaI polymorphism were associated with obesity (5.5 % vs 3.9%, $p=0.44$; 2.5% vs 2.0% $p=0.68$; 15.3% vs 18.6%, $p=0.35$; 30.9% vs 31.8%, $p=0.84$, for allele frequencies of ins56, Glu188Gly, C8246T and RsaI in control and obese groups) or BMI. Lean carriers of the 8246-CC genotype had higher serum leptin levels compared to CT/TT carriers (9.7 ± 6.6 vs 6.7 ± 4.4 µg/l, $p=0.036$), especially lean females (13.6 ± 5.8 vs 8.5 ± 4.4 µg/l, $p=0.0021$), and lean female carriers of a C8246T(CC)/RsaI(-) haplotype (14.9 ± 4.5 vs 10.8 ± 5.7 µg/l, $p=0.0017$). Neither the C8246T variant nor the C8246T(CC)/RsaI(-) haplotype were associated with serum leptin levels in obese subjects (32.4 ± 12.2 vs 33.7 ± 11.9 µg/l, $p=0.62$ and 33.4 ± 11.9 vs 32.7 ± 12.4 µg/l, $p=0.79$). **Conclusions:** Monogenic forms of obesity due to mutations in POMC and MC4R are rare in Swedish obese patients. POMC gene variants contribute to normal variations in leptin levels in lean but not in obese individuals.

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Is a low level of plasma leptin in severe female obesity associated with metabolic risk factors?

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Background and Aims: Leptin is multifactorially regulated and following weight reduction (WR) leptin is reduced. We speculate if differences in leptin before and following WR in severe obesity are associated with a metabolic profile compatible with an increased cardiovascular risk.

Materials and Methods: 13 female non-diabetic patients, aged 34 ± 4 yrs with body mass index (BMI) ranging from 40.5 to 57.0 (mean 46.4 ± 5.5) were studied before and after bariatric surgery. 2-hr glucose was assessed with OGTT. Fasting levels (at 08.00) of p-leptin, triglyceride (TG), HDL cholesterol, insulin and HOMA IRI (homeostasis model assessment of insulin resistance index) were measured before and after 25% WR. Insulin secretion (AIR) was studied after iv arginine (5g) at fasting glucose and at p-glucose (PG) levels of 14 and >25 mmol/l.

Results: At 7-15 months after bariatric surgery, BMI was reduced to 35.0 ± 5.7 kg/m² ($p<0.001$), leptin from 67.5 ± 17.2 to 20.5 ± 11.0 ng/ml ($p<0.001$), TG from 2.0 ± 1.2 to 1.0 ± 0.4 mmol/l ($p<0.01$), 2-h glucose from 7.6 ± 0.4 to 5.5 ± 0.2 mmol/l ($p<0.01$), insulin from 146 ± 1 to 63 ± 5 pmol/l, ($p<0.01$) and HOMA IRI from 35 (18-44) to 11 (8-21), ($p<0.005$). There were no alterations in WHR (from 0.82 ± 0.04 to 0.85 ± 0.1 , $p=NS$) and HDL (from 1.1 ± 0.4 to 1.2 ± 0.3 mmol/l, $p=NS$). In relationship there was clearly a positive correlation with TG and 2-h glucose ($r=0.67$, $p<0.02$ and $r=0.61$, $p<0.04$; before and after WR resp.), and with delta TG and delta AIR at PG >25 mmol/l ($r=0.75$, $p<0.02$). Leptin showed an inverse correlation with WHR ($r=-0.65$, $p<0.02$ and $r=-0.65$, $p<0.03$; before and after WR resp.) and a direct association with HDL cholesterol ($r=0.76$, $p<0.01$ and $r=0.67$, $p<0.048$; before and after WR resp.). Leptin had a tendency to correlate inversely with TG before WR ($r=-0.55$, $p<0.077$). The reduction of leptin was inversely associated with the reduction of TG ($r=-0.67$, $p<0.03$). In a forward stepwise regression model to predict delta leptin, delta HOMA IRI and delta insulin were by far the strongest factors ($p<0.0079$ and $p<0.01$, resp.), (whole model R square 0.95).

Conclusions: Our results suggest that in severe obesity a low level of leptin is related to metabolic risk factors that is only partly compensated after 25% WR. The alterations in leptin is highly dependent of alterations in insulin resistance. The relationship between TG and 2-h glucose and TG and AIR, suggest a role for TG in the deterioration in glucose tolerance as well as stimulation of maximal insulin secretion.

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Other Hormones

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THE EFFECT OF GROWTH HORMONE ON GLYCOGEN SYNTHESIS IN PRIMARY RAT HEPATOCYTES

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Background and Aims: Hepatic glycogen synthesis is reported to be increased in hypophysectomized rats, and the aim of this study was to investigate if GH has any effect on hepatic glycogen synthesis, and to elucidate if the putative effect of GH is a direct effect on hepatocytes or mediated through the increased availability of free fatty acids (FFAs) from adipose lipolysis in animals treated with GH. **Materials and Methods:** Primary hepatocytes were isolated from male and female Sprague Dawley rats and cultured. Glycogen synthesis was induced with insulin and glucose \pm GH and/or FFAs. Insulin binding was performed in the presence or absence of either GH or FFAs (palmitic acid). **Results:** The glycogen synthesis in the hepatocytes was stimulated by GH when incubated for 20 hours, whereas no effect of GH was observed after only 4 hours. Furthermore, GH potentiated the effect of insulin on glycogen synthesis in male and female hepatocytes incubated for 4 and 20 hours. To examine this effect we looked for a change in insulin receptor binding. In accordance with the observed effect, incubation of the hepatocytes with GH for 20 hours resulted in a significant increase in specific binding of insulin, corresponding to an increase in Bmax values (female: 27.4 ± 0.8 vs. 33.1 ± 0.8 ; male: 23.1 ± 0.6 vs. 28.9 ± 1.1 fmol/mg protein). The stimulating effect of GH on insulin binding sites and insulin-stimulated glycogen synthesis after 20 hours is speculated to be associated with an effect of GH on de novo protein synthesis of the IGF-I and/or the insulin receptor. The effect of GH on glycogen synthesis in the absence of insulin might be a result of increased IGF-I synthesis and release, resulting in an interaction of IGF-I with the insulin receptor. GH's ability to stimulate insulin-induced glycogen synthesis at 4 hours was speculated to be a direct effect through increased phosphorylation of insulin receptor substrate 2. When hepatocytes were incubated with FFAs GH and insulin-stimulated glycogen synthesis was decreased. We tested, if the insulin binding was altered and we showed that male hepatocytes pre-incubated with FFAs for 3 hours exhibited a reduced insulin binding corresponding to a reduction in the number of receptors (29.36 ± 1.5 vs. 20.1 ± 0.5 fmol/mg protein). FFAs have previously been shown to reduce insulin-stimulated glucose uptake in muscle, and here we show that they also affect hepatic glycogen synthesis by decreasing. **Conclusions:** The study shows that GH regulates hepatic insulin sensitivity in primary hepatocytes, measured as glycogen synthesis, both directly and indirectly (through FFAs) by regulating the amount of insulin receptors.

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TAURINE REDUCES GLUCOSE METABOLISM DISORDER INDUCED BY GROWTH HORMONE IN RATS

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Abstract Background and aims: To investigate whether the taurine (Tau) can prevent the glucose metabolic disorder induced by growth hormone (GH) in the rats. **Materials and Methods:** Male rats were randomly separated into groups receiving GH, GH+Tau, Tau and vehicle (n=7, respectively). The rats in GH group were treated with 1mg GH injection, twice a day, for 5 days; the rats in GH+Tau group, apart from 1mg GH injection, were treated with feed water including 2% Tau. Oral glucose tolerance test was given to all the rats in each of the groups at the 6th day. Insulin releasing stimulated by glucose, skeletal muscle glycogen synthesis, endothelin (ET) and nitric oxide product (NO₂) were examined. **Results:** The basal and 30-min post glucose tolerance glucose level was significantly higher in GH group than that in control (vehicle) group. The insulin levels before and after glucose load were significantly increased in GH group when they were compared those in control group (basal: 17.17 ± 3.44 vs 8.19 ± 1.03 , $p<0.01$; 30': 38.88 ± 5.02 vs 19.73 ± 2.80 , $p<0.01$; 60': 29.23 ± 8.15 vs 13.23 ± 3.58 , $p<0.01$; 120': 13.16 ± 3.16 vs 8.01 ± 1.63 mmol/L, $p<0.05$). However, the decline of skeletal muscle glycogen synthesis in GH group (GH: 17.05 ± 1.96 vs Con: 22.69 ± 3.08 , nmol/mg ww; $p<0.01$), was especially evident when insulin was present (GH: 49.60 ± 9.18 vs Con: 95.85 ± 5.75 , nmol/mg ww; $p<0.01$). GH treatment resulted in a higher level of plasma ET compared with that treated with vehicle in control group. The taurine treatment significantly improved the above changes, and the glycogen synthesis stimulated by insulin was increased more than that in GH group (70.3 ± 8.31 vs 49.60 ± 9.18 nmol/L ww; $p<0.01$) in particular. **Conclusion:** The taurine treatment could reduce the glucose metabolism disorder induced by GH treatment.

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GH RESPONSE TO GHRH + GHRP-6 DURING EUGLYCEMIC AND HYPERGLYCEMIC CLAMP IN TYPE 2 DIABETES MELLITUS

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Background and Aims: Conflicting results have been reported on the growth hormone (GH) response to GH secretagogues in patients with type 2 diabetes mellitus. The aim of our study was to investigate the GH response to GHRH + GHRP-6 during euglycemic (EC) and hyperglycemic clamp (HC) in type 2 diabetic patients.

Materials and Methods: 12 male patients with type 2 diabetes (mean age: 54.08 ± 1.45 years; BMI: 25.58 ± 0.39 kg/m²; mean HbA1c: 8.79 ± 0.42%) were investigated. GH (fluoroimmunoassay, Delfia, mcg/l) response to stimulation with GHRH (GRF 1-29 NH₂, Geref, Serono, Spain; 100 mcg i.v.) plus GHRP-6 (His-D Trp-Ala-Trp-D Phe-Lys-NH₂, Peninsula Labs, Heyerside, UK; 90 mcg i.v.) was measured at -30; -15; 0; 15; 30; 45; 60; 90 and 120 min. GH was measured twice in the same patients, first during EC and week later during HC. Each clamp lasted 4 hours and GH response was measured in last two hours of the clamps. Data are presented as the mean ± SEM. The areas under the secretory curve (AUC) for GH (mcg/l/120 min) was calculated by a trapezoidal method. Wilcoxon test was used for statistics.

Results: Mean glycaemia in EC was 4.93 ± 0.05 mmol/l, in HC: 12.19 ± 0.07. Basal GH did not differ between EC and HC (2.9 ± 0.99 vs. 1.48 ± 0.44 mcg/l, p > 0.05). Peak GH was higher in EC than in HC (151.06 ± 16.86 vs. 112.45 ± 14.45, p < 0.05). AUC for GH in EC was higher than in HC (9560.75 ± 1140.64 vs. 6974.49 ± 1001.95, p < 0.05).

Conclusions: GH response to GHRH + GHRP-6 is significantly lower in patients with type 2 diabetes during hyperglycemic clamp in comparison with euglycemic clamp, indicating significant influence of glucose levels on GH response to applied secretagogues.

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BEACON, A NOVEL PEPTIDE – MAY HAVE A CENTRAL AND PERIPHERAL ROLE ON ENERGY METABOLISM.

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Background and Aims: Beacon is a novel gene discovered by differential display PCR as overexpressed in the hypothalamus of obese vs. lean Israeli Sand Rats (*Psamomys obesus*), a unique animal model of obesity. Beacon gene expression in the hypothalamus was shown to be positively correlated with percent body fat. When administered by ICV, a chemically synthesized peptide corresponding to N-terminal 33 amino acids of Beacon protein increased the food intake and bodyweight of animals. Here we report the findings on Beacon protein expression in different tissues using recently made polyclonal antibodies.

Materials and Methods: The 219 bp long cDNA coding for full length (putative 73aa) beacon was cloned into a pGEX2T vector, expressed and purified as a GST-fusion protein. The GST tag was removed by Thrombin cleavage and the contaminating Thrombin was removed by treatment with benzamidine-sepharose. Rabbits were immunized with GST-beacon or cleaved beacon conjugated to diphtheria toxoid. Beacon specific antibodies were affinity purified using beacon immobilized on Aminolink or Tetralink resin. Beacon expression in various sandrat tissues was analyzed using affinity purified anti-beacon antibodies.

Results: Recombinant GST-beacon and GST-free beacon protein samples produced were homogeneous (>95% pure). Affinity purified antibodies facilitated qualitative expression analysis of endogenous beacon protein. Expression of Beacon in a wide variety of tissues, in the expected molecular size was observed.

Conclusions: Results implicate a role for beacon in the central as well as peripheral tissues. An understanding of the precise nature of these roles and their impact on the control mechanisms involved in energy homeostasis will require further studies.

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Distinct Responses to Glucose of Free ATP Concentration Imaged Dynamically in Hypothalamic Neurons and Glial cells.

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Background and Aims: Changes in the activity of neurons in the ventro-medial and arcuate nuclei of the hypothalamus are important for regulating feeding and satiety. These cells respond to changes in blood glucose and leptin concentrations either with increases or decreases in electrical activity, though the molecular mechanisms involved are unclear. Since increases in the ratio of free ATP / ADP concentration in islet β-cells are considered important for detecting changes in blood glucose concentrations, we determined whether similar changes may be involved in hypothalamic neurons.

Materials and Methods: Hypothalamic regions were isolated from 2-4 day old rat pups, dissociated into individual cells and cultured for 10 days, before 48 h infection with adenovirus expressing cytosol-targeted recombinant firefly luciferase. Changes in intracellular [ATP] were monitored by imaging bioluminescence with an inverted optics microscope (20x objective) and ultra-sensitive charge-coupled device camera.

Results: Glucose-responsive neurons were morphologically distinct from other neurons and glial cells after 10 days culture. Transfer of the luciferase gene into both cell types was efficiently achieved with the adenoviral vector. Stepped increases in glucose concentration from 0 to 3 and 3 to 15 mM glucose had no significant effect on light output from glucose-responsive neurons (-0.7 ± 4.0 % and +0.5 ± 5.1 %, respectively; n = 20 cells from 3 separate preparations). However, neighbouring glial cells gave a small increase in response to 3 mM glucose (+1.8 ± 1.6 %; n = 68) but a large increase at 15 mM glucose (+12.5 ± 2.3 %; p < 5 % vs. neurons).

Conclusions: These data suggest that nutrient sensing by hypothalamic neurons may be different from that in pancreatic β-cells. In particular, changes in intracellular [ATP] in glial cells may play a previously unsuspected role in the regulation of hypothalamic neurons by glucose, and thus in feeding behaviour.

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EFFECT OF α-MELANOCYTE STIMULATING HORMONE ON GLUCOSE TRANSPORT IN RAT ADIPOCYTES

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We have previously reported that the plasma levels of α-melanocyte stimulating hormone (α-MSH) are significantly increased and that they are correlated with insulin resistance in obese men. **Aims:** To assess the direct effect of α-melanocyte stimulating hormone on insulin-stimulated glucose transport and on translocation of insulin-regulatable glucose transporter (GLUT4) in rat adipocytes. **Material and Methods:** Adipocytes were isolated from epididymal adipose tissue from Sprague-Dawley rats. Melanocortin receptor expression in adipocytes was examined by RT-PCR analysis. Adipocytes were incubated with or without various concentrations of Nle⁴, D-Phe⁷-α-MSH, a synthetic α-MSH agonist, followed by incubation with or without 10nM insulin. The glucose transport activity was measured by determining the uptake of 3-O-[methyl-³H]-D-glucose, and the translocation of GLUT4 was measured by immunoblot. **Results:** Melanocortin 5 receptor was detected in rat adipocytes by RT-PCR. Nle⁴, D-Phe⁷-α-MSH did not affect the basal glucose transport but reduced the insulin-stimulated glucose transport in adipocytes in a dose-dependent manner. About 45% of inhibition was observed with 1nM Nle⁴, D-Phe⁷-α-MSH under insulin stimulation. Nle⁴, D-Phe⁷-α-MSH did not affect the insulin-stimulated translocation of GLUT4 from the intracellular pool to the plasma membrane. **Conclusion:** Adipocytes express melanocortin 5 receptor. α-MSH reduced insulin-mediated glucose transport at the post-receptor level without affecting the translocation activity of GLUT4 from the intracellular pool to the plasma membrane in rat adipocytes.

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IMPACT OF DIETARY IMPROVED GLUCOSE CONTROL ON SEX HORMONES IN PATIENTS WITH TYPE 2 DIABETES.

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Background and Aims: Evidence for an association between sex hormones and insulin sensitivity is provided by several circumstances with a development of insulin resistance in response to inappropriate sex hormone levels, e.g. women with hyperandrogenic conditions, such as polycystic ovary syndrome, the surgically postmenopausal cynomolgus monkey, anabolic steroid abusers and transsexuals under testosterone or ethinyl estradiol treatment. After several cross-sectional studies have documented inappropriate sex hormone levels in female and male patients with type 2 diabetes (decreased androgens in males and increased androgens in females, low SHBG in males and females), we have now investigated the effect of dietary improved glucose control on sex hormones in such patients.

Materials and Methods: 72 subjects with type 2 diabetes were studied before and after four weeks of moderate caloric restriction (47M/25F; age: 63±1 years (38-79 years); duration of diabetes: 5.2±0.7 years; HbA_{1c}: 7.7±0.2 % - mean±SEM). None of the patients required insulin therapy nor was positive for antibodies against glutamate decarboxylase, insulin or islet cells.

Results: A mean weight loss of 2.3±0.3 kg ($p < 0.0001$; M: 2.5±0.4 kg; F: 1.8±0.3 kg) was associated with a significant improvement of glucose homeostasis: HbA_{1c}: -0.5±0.1 % ($p < 0.0001$); fasting glucose: -12±5 mg/dl ($p = 0.003$); postprandial glucose: -23±6 mg/dl ($p = 0.001$). Insulin and c-peptide as well as 17β-estradiol concentrations remained unchanged. Testosterone levels fell in the female patients (-5±2 ng/dl, $p = 0.018$), whereas testosterone concentrations significantly rose in the male subgroup (56±25 ng/dl, $p = 0.028$). Significant changes were also documented for DHEAS (-119±42 ng/ml, $p = 0.004$) and SHBG (+5.5±2.1 nmol/l, $p = 0.034$), but only in the female subgroup.

Conclusions: Dietary control of glucose metabolism improves inappropriate androgen levels in female and male patients with type 2 diabetes.

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Age dependent decline of serum testosterone and its potential implication for general and sexual well being in diabetic men.

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Background and Aims: Men with diabetes have a high prevalence of sexual dysfunction. Data concerning the age-trend of testosterone in men with diabetes compared to healthy men and its importance for general and sexual well being in diabetic men are conflicting. The aim of the study was to compare a large group of patients with recently diagnosed type 2 diabetes mellitus to healthy controls.

Materials and Methods: Serum levels of free testosterone were determined in diabetic men ($n = 132$; age: mean = 61±9.4; 30-79 ys) and compared to serum levels of healthy controls between 20-80 years of age ($n = 572$). In addition, in diabetic men general and sexual well being were assessed by standardised questionnaires and the results were investigated with respect to serum levels of testosterone, medication and comorbidity.

Results: Mean serum levels of testosterone were lower in diabetics than expected for the same age in healthy men ($p < 0.001$). There was a slight but significant steeper age trend for testosterone in diabetic as compared as to healthy men. In diabetic men testosterone levels were positively associated with self-rating scores of constructs of sexual function ($p < 0.01$) and libido, but not with constructs of general or social well being. Serum levels of free testosterone did differ between men treated with insulin and men controlled with oral antidiabetics ($p < 0.001$). Cardiovascular comorbidity was predictive for lower levels of testosterone and lower scores in sexual and general well being ($p < 0.001$).

Conclusions: The study demonstrates a more rapid decline of testosterone with age in diabetic as compared as to healthy men. This might be aggravated if cardiovascular comorbidity is present. The results confirm earlier reports on the association between low androgen levels and sexual dysfunction and complaints in diabetic men.

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HYPOTHALAMIC-PITUITARY-TESTICULAR AXIS (HPTA) FUNCTION IN WELL-CONTROLLED AND POORLY-CONTROLLED TYPE 1 DIABETIC MEN

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Background and Aims: The impact of poor glycemic control on the HPTA function in patients with type 1 diabetes-mellitus (DM-1) is unknown. We analyzed the function of HPTA in uncomplicated DM-1 patients.

Materials and Methods: Eleven poor controlled (P-C) (HbA_{1c} >10%) and 8 well controlled (W-C) (HbA_{1c} <8%) DM-1 patients (both with <7 yr of DM-1), and 9 age- and BMI-matched controls, underwent blood sampling at 10-min intervals for 10 h before and after consecutive iv administration of two 10 ug doses of gonadotropin-releasing hormone (GnRH). Leydig cell function was assessed by measuring serum testosterone (T) before and after a single im administration of choriogonadotropin (hCG) (40 IU/kg).

Results: Baseline levels of LH in samples collected during a 6-h period were significantly decreased in P-C (12±4 IU/L; mean±SEM) as compared to W-C (19±5) and controls (19±5). The magnitude of the LH response to the first GnRH stimulus was similar among groups, whereas the response to the second stimulus was significantly decreased in P-C [Δ LH=48±7 IU/L, area under the curve (AUC)=8342±958 IU/L/min] compared to W-C (Δ LH=92±13 IU/L, AUC=8342±958 IU/L/min) and controls (Δ LH=93±19 IU/L, AUC=7671±1275 IU/L/min) ($p = 0.029$ and $p = 0.031$, respectively). Although baseline T levels were decreased in P-C subjects (21±3 nmol/L), the difference between these levels and those exhibited by W-C (29±2) and controls (26±2) was not statistical significant; the early (6h) and late (3 days) response to hCG were also similar among groups, albeit in the P-C group the late response was significantly ($p = 0.04$) increased (2.0±0.2 times to basal vs 1.5±0.1 times in W-C and control subjects).

Conclusions: These data indicate that subtle abnormalities in HPTA function (from hypothalamic origin), are present in patients with DM-1 during periods of poor glycemic control. (Study supported by a CONACyT award to JC LA No. 116515)

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DIFFERING EFFECTS OF GLUCOCORTICOIDS ON METABOLIC AND VASCULAR INSULIN SENSITIVITY

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Background: Previous studies have shown a relationship between insulin action in metabolic and vascular tissues. Common intracellular signalling intermediaries have been identified in adipose tissue, muscle and the endothelium, providing a potential mechanism through which insulin action in these tissues may be linked. We hypothesised that modification of metabolic insulin sensitivity by glucocorticoid administration would be accompanied by similar changes in insulin action in the vasculature. Aim: To examine whether low dose dexamethasone administration is associated with a reduction in both metabolic and vascular insulin sensitivity.

Methods: 20 healthy volunteers were recruited into a double-blind, randomised placebo controlled trial, consisting of two six-day phases of oral dexamethasone or matched placebo. The ethics committee of the West Glasgow Hospitals University NHS Trust approved all aspects of the study. After each phase, volunteers underwent buttock biopsy to obtain small resistance arteries (200-400mm internal diameter), followed by a euglycaemic hyperinsulinaemic clamp. The vessels were mounted on a wire myograph and underwent a standard normalisation protocol prior to assessment of ex vivo insulin-mediated attenuation of norepinephrine-induced vasoconstriction.

Results: Dexamethasone treatment was associated with a reduction in insulin sensitivity (M value on placebo 10.12 ± 0.45 mg/kg/min vs. 7.2 ± 0.55 mg/kg/min on dexamethasone, $p < 0.01$). During both phases, pretreatment with physiological insulin concentrations resulted in vasodilation; there was no difference in magnitude between study phases.

Conclusions: Despite glucocorticoid administration causing a substantial reduction in whole body insulin sensitivity, there was no associated reduction in the response to insulin in the vasculature. This suggests that, in this model, metabolic and vascular insulin sensitivity are differentially regulated.

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Dexamethasone impairs IRS-1, PI 3-kinase and Protein kinase B expression as well as glucose transport and this occurs independent of glucose and insulin levels.

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Background and aims: Excess endogenous secretion or exogenous administration of glucocorticoids promotes insulin resistance. This study explores the effects of glucocorticoids on the glucose transport system in rat adipocytes and the interaction with high levels of insulin and glucose. **Materials and Methods:** Isolated rat adipocytes were cultured for 24 h at different glucose concentrations (5 and 15 mM) with or without the glucocorticoid analog dexamethasone (0.3 μ M) and insulin (10,000 uU/ml). After the culture period, the cells were washed and then basal and insulin-stimulated ¹⁴C-glucose uptake, ¹²⁵I-insulin binding as well as cellular content of insulin signaling peptides (IRS-1, IRS-2, PI 3-kinase and PKB) and GLUT4 were measured. **Results:** Dexamethasone in the medium markedly decreased both basal and insulin-stimulated glucose uptake compared to control cells at both 5 and 15 mM glucose (by ~40-50 %, $p < 0.001$ and $p < 0.05$, respectively). Combined long-term treatment with insulin and dexamethasone exerted additive effects in decreasing glucose uptake (by ~50 % compared to dexamethasone alone, $p < 0.01$), but this was seen only at 15 mM glucose and not at 5 mM. Furthermore, insulin binding was decreased (by ~40 %, $p < 0.05$) in dexamethasone-treated cells independent of surrounding glucose concentration. Following dexamethasone treatment a ~75 % decrease ($p < 0.001$) in IRS-1 protein abundance in total cell lysates and an increase in IRS-2 (by ~150 %, $p < 0.001$) was found. Dexamethasone also induced a subtle but consistent decrease in PI 3-K (by ~15 %, $p < 0.01$) and a substantial decrease in PKB (by ~45 %, $p < 0.001$). Dexamethasone did not alter the amount of total cellular membrane-associated GLUT4 protein. The effects of dexamethasone per se on glucose transport and insulin signaling peptides were mainly unaffected by the surrounding glucose and insulin levels. **Conclusions:** Glucocorticoids impair glucose transport capacity in fat cells independent of the surrounding glucose and insulin concentration. This is not due to alterations in GLUT4 abundance, but the demonstrated decrease in the insulin signaling peptides IRS-1 and PKB may play an important role. Decreased insulin binding capacity might contribute to a reduced insulin sensitivity of the glucose transport system.

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Epinephrine stimulates insulin secretion in a Ca²⁺/calmodulin-dependent protein kinase II δ 2 deficient rat insulinoma cell line INS d-W12J. Schumacher, M. Osterhoff, A.F.H. Pfeiffer
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Background and Aims: In normal INS-1 cells Ca²⁺/calmodulin-dependent protein kinase II δ 2 (CaMK II δ 2) is the major subtype. We reported about a CaMK II δ 2 deficient cell line called INS d-W12 previously which we obtained from INS-1 cells by a stable antisense RNA approach. In the present work we investigated the effect of various α 1-, α 2- and β -receptor agonists and antagonists on the glucose-dependent insulin secretion in INS-1 and INS d-W12 cells.

Materials and Methods: INS-1 rat insulinoma cells and the antisense cells INS d-W12 were cultured at 37 °C, 5% CO₂ in RPMI 1640 media containing 10% FBS, 2 mmol/l L-glutamine, 2 mmol/l pyruvate and 1 mmol/l 2-mercaptoethanol. They were pre-incubated in Ca²⁺-containing Krebs-Ringer-buffer for 2 h and finally treated with different stimulants for 1 h in Ca²⁺-containing Krebs-Ringer-buffer. The secreted insulin was measured in the media by insulin RIA.

Results: The INS d-W12 cells revealed a rather unexpected behavior concerning epinephrine-dependent insulin secretion. While in normal INS-1 cells epinephrine at a concentration of 10 μ mol/l suppressed the glucose-dependent insulin secretion it was increased in INS d-W12 cells ($p = 0.004$). Pertussistoxin (PTX) an inhibitor of G₀ and G_i blocked the α 2-mediated epinephrine effect in INS-1 cells but had no effect on INS d-W12 cells. Propranolol a β -receptor antagonist had no effect on the insulin secretion in both INS-1 and INS d-W12 cells. Clonidine an α 2-receptor agonist was able to inhibit insulin secretion in INS-1 cells by 30% at concentrations of 1 μ mol/l ($p = 0.002$) but had no effects in INS d-W12 cells insulin secretion was not inhibited.

Conclusions: These findings show that epinephrine unexpectedly stimulates insulin secretion in INS d-W12 cells while they confirm the known inhibitory effect via α 2-receptors in INS-1 cells. The stimulatory effect did not involve β -receptors as shown by propranolol and was apparently mediated by a PTX independent pathway.

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ACUTE HYPERCORTISOLEMIA INCREASES INTERSTITIAL GLYCEROL IN SUBCUTANEOUS ADIPOSE TISSUE

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Background and Aims: The glucoregulatory hormones, cortisol, epinephrine, glucagon and growth hormone (GH), control fuel homeostasis in the fasting state. FFA dominates the energy supply to resting striated and cardiac muscle as well as to the kidneys and liver. Whether cortisol similar to GH and epinephrine possesses lipolytic actions is however still controversial

Materials and Methods: We applied the recently introduced microdialysis technique in 7 healthy lean males after an overnight fast in a placebo controlled randomised cross-over design with either hydrocortisone or saline infusion. Two microdialysis catheters were inserted at t=-150 min and perfused with Ringer solution. At t=-120 min a pancreatic-pituitary clamp was initiated using somatostatin (330 μ g/h), insulin (0.08 mU/kg/min), GH (2 ng/kg/min) and glucagon (0.5 ng/kg/min). Plasma glucose was maintained above 4.5 mmol/l by glucose infusion. At t=0 infusion of either saline or hydrocortisone was commenced. Arterialised blood samples were drawn every 30 min and analysed for FFA, glycerol and hormonal concentrations.

Results: During hydrocortisone infusion serum cortisol was more than threefold higher as compared to placebo (888 \pm 12 vs. 245 \pm 7 nmol/l). Similarly serum FFA and blood glycerol were significantly elevated during hydrocortisone exposure (0.612 \pm 0.01 vs. 0.320 \pm 0.01 mmol/l; $P = 0.015$ and 51 \pm 3 vs. 34 \pm 3 mmol/l; $P = 0.054$). Substantial increase in interstitial glycerol was seen during hypercortisolemia in both abdominal (320 \pm 11 vs. 150 \pm 9 nmol/l; $P = 0.046$) and femoral (169 \pm 9 vs. 99 \pm 6 nmol/l; $P = 0.047$) subcutaneous adipose tissue.

Conclusions: The present study supports the notion that high physiological cortisolemia exerts lipolytic properties in subcutaneous adipose tissue.

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INTESTINE-SPECIFIC ACTIVITY OF THE GLUCOSE-6 PHOSPHATASE GENE PROMOTER IS REGULATED BY THE HOMEODOMAIN PROTEIN CDX1.

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Background and aims: Glucose-6 phosphatase (G6Pase) confers on gluconeogenic tissues the capacity to release glucose in blood. The expression of its gene is restricted to liver, kidney and small intestine, and is increased during diabetes and fasting, in the three tissues. The aim of this study was to identify transcription factors involved in the intestine-specific expression. **Materials and Methods:** The -1640/+60 bp G6Pase gene region was cloned upstream of a luciferase reporter gene. G6Pase promoter activity was assessed by transient transfections in intestinal CaCo-2 cells, hepatoma HepG2 cells, and in HeLa cells. **Results:** We previously showed that G6Pase gene was expressed in HepG2 cells and in enterocyte-differentiated CaCo-2 cells (post-confluence cultures) but not in undifferentiated CaCo-2 and in HeLa cells. All promoter constructs (from -1640/+60bp to -80/+60bp) were highly active in HepG2 cells but have no activity in HeLa cells. A very weak promoter activity was detected in undifferentiated CaCo-2 cells. However, the co-expression of hepatocyte transcription factors, HNF1 or HNF4, was sufficient to restore a high G6Pase promoter activity in HeLa and CaCo-2 cells. The expression of the cdx1 (caudal-related homeodomain protein specifically expressed in intestine) recombinant protein, in HNF4- and/or HNF1-expressing HeLa cells, repressed the G6Pase activity of the fragments -1640/+60bp to -160/+60bp. There was a very weak (2-fold) induction of the fragment -80/+60 bp activity. In contrast, cdx2 did not modify the promoter activity of any construct. In bandshift assays, we showed that cdx1 and cdx2 bind to the TATA box. The mutation of the TATA box in the -500/+60bp promoter construct resulted in the activation of the G6Pase promoter in HeLa cells. **Conclusions:** These results indicate that the binding of cdx1 on the TATA box is critical for the inhibition activity of the distal region on the G6Pase promoter. Because HNF1 and HNF4 are also expressed in enterocytes, cdx1 could be the intestinal factor involved in the down-regulation of the expression of the G6Pase gene in enterocytes, accounting for the lower G6Pase expression in enterocytes as compared to hepatocytes.

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Effects of insulin on glucose uptake and glycogen synthesis in the perfused rat liver. Possible role of gluconeogenesis.

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Background and Aims: Insulin is a major stimulatory factor in the synthesis of hepatic glycogen. Glycogen synthesis has been linked to the parallel formation of glucose-6-phosphate by gluconeogenic pathways (g6p-neogenesis). However insulin is also considered an inhibitor of gluconeogenesis which would not be consistent with this observation. The effects of insulin on these processes were therefore examined in the perfused rat liver.

Materials and Methods: Fifteen 24h-fasted Sprague-Dawley rats were donors of livers for recirculating perfusions (11.5ml/min) using erythrocytes in plasma from donor rats. Initial glucose levels were ~6mmol/l, [6-3H]glucose was added and lactate (1mg/min) along with [U-14C]lactate was infused continuously for 120min. Insulin was infused to maintain levels of 0, 70 and 135pmol/l (n=5 each). Total and gluconeogenic glucose production was determined from inflow - outflow differences of glucose and [14C]glucose, and lactate specific activity. Glucose uptake was determined from differences in [6-3H]glucose. Total and labeled glycogen was measured in freeze-clamped liver biopsies at the end of the perfusion. Direct and gluconeogenic fractions were estimated from the incorporation of glucose label and gluconeogenic flux to glucose-6-phosphate from the glucose uptake, production and these fractions. Statistical comparisons used one-way analysis of variance.

Results: Actual insulin concentrations were 0, 77±14 and 189±35 pM. Glucose fell from 12.8±0.5 to 9.8±0.8 mM at the higher dose (p<0.05). Lactate levels did not change with insulin dose. The fraction of glycogen synthesis that arose directly from glucose was 0.4 and remained unchanged by insulin (p>0.5). Mean glucose uptake over 120min was 2.6±0.4, 3.0±0.9 and 4.6±0.5 µmol/min with increasing insulin. G6p-neogenesis also increased from 1.51±0.04 to 1.85±0.21 mmol over 120min (p<0.05), paralleled by an increase in glycogen from 21±3 to 40±6 mg (p<0.05). Modeling the relationship between [14C]-labeled glucose, lactate and glycogen, further indicated that the flux from lactate to glucose is unchanged by insulin, suggesting that the increase in glucose uptake accounts for most of the change in perfusate glucose.

Conclusions: Since the highest insulin dose used, corresponds to the ED50 of the effect of insulin on glucose production by the liver, these data suggest that at non-saturating, suprabasal insulin concentrations, gluconeogenic flux to glucose-6-phosphate continues unabated and appears to be stimulated. This increase parallels the increases in glucose uptake and glycogen synthesis and may contribute to the determination of these fluxes.

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REGULATION OF INTESTINAL GLUCOSE PRODUCTION IN LONG - TERM FASTED RATS

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Background and Aims: Glucose-6 phosphatase (Glc-6Pase) is the last enzyme of gluconeogenesis and glycogenolysis. It is only expressed in the liver, the kidney and the small intestine (SI) and confers on these organs the capacity to produce glucose in blood. SI Glc-6Pase is induced in diabetic and 48h-fasted rats. In both situations, it has been shown that significant intestinal glucose production (IGP) takes place. We have tested the hypothesis that IGP is correlated with the Glc-6Pase gene induction during fasting in rats.

Materials and Methods: Fed postabsorptive and 24 -, 48 -, 72 - and 96h-fasted rats were anesthetized and infused by 3-3H Glucose for 90 min. IGP and total endogenous glucose production (EGP) have been determined using a combination of arteriovenous balance and isotopic dilution techniques, from the specific activities and concentrations of glucose in arterial and portal blood. Glc-6Pase activities were determined in jejunum fragments, which were rapidly sampled and frozen at -196 C. The respective abundances of Glc-6Pase and phosphoenolpyruvate carboxykinase (PEPCK) mRNAs were quantified using northern blot.

Results: In fed and 24h-fasted rats, SI did not release glucose in blood. Glc-6Pase activity was not significantly altered in SI in both groups. After 48h of fasting, IGP was 8.5 ± 1.4 µmoles/kg/min (mean ± SD, n=8), representing 21% of total EGP. IGP was maximum in 72h-fasted rats : 19.2 ± 4.8 µmol/kg/min (n=8), plateauing at 96h. This represented 40% of EGP. After 48, 72 and 96h of fasting, SI Glc-6Pase activity was markedly increased by 2-3 fold. At the mRNA level, the expression of Glc-6Pase and PEPCK genes was strongly increased from 24h of fasting, and was maximum (12 fold) after 48h up to 96h of fasting.

Conclusions: Our results strongly suggest that the induction of the Glc-6Pase gene plays a crucial role in the induction of IGP. Further investigations of the mechanisms of regulation of IGP will likely allow a better understanding of the mechanisms by which EGP is increased in type 2 diabetes.

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EFFECTS OF ORAL METAVANADATE TREATMENT ON LIVER GLUCOSE-6-PHOSPHATASE IN BB RAT

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Background and Aims: Glc6Pase - a multifunctional enzyme involved in the last step of gluconeogenesis and glycogenolysis is thought to play an important role in glucose homeostasis. The aim of this study was to investigate the long-term effect of vanadate treatment on the hydrolytic and phosphotransferase activities of liver Glc6Pase in diabetic and non-diabetic BB rats. **Material and Methods:** BB-DP rats (mean aged 40 days) divided into 4 similar groups - V1, V2, V3 and C - have received three doses of NaVO₃ (0.1, 0.2 and 0.7 mg/ml) and respectively NaCl (0.5 mg/ml) in drinking water for 7 days. After this treatment, all animals were monitored over a 90-day period for changes in weight, glycemia and glucosuria to detect onset of diabetes. Hydrolytic and phosphotransferase activities of Glc6Pase were assayed as described by Nordlie and Aron [Methods in Enzymology Vol.9, pp. 619-625, 1965] in isolated microsomes. The assays were made in the beginning and the end of vanadate treatment and after 83 days. **Results:** Some toxic effects in BB rats treated with 0.7 mg/ml metavanadate were noticed. Treatment caused a reduction in incidence of diabetes, increasing the age when the rats become diabetic, proportionally with dose applied. The Glc6Pase and CP:GlcPTase activities were 0.99±0.12 and 0.16±0.03 µmoles/min/mg protein respectively, in non-diabetic BB rats before treatment. After diabetes onset, the hydrolase and transferase activities were elevated 3.8 and 2.2 fold, respectively. Treatment with metavanadate decreased the both activities, proportionally with dose applied but was more potent inhibitor of CP:GlcPTase activity. After 90 days in non-diabetic animals, the Glc6Pase activity is restored but the CP:GlcPTase activity remain increased (0.35 ± 0.04). **Conclusions:** Elevated CP:GlcPTase after 83 days from the treatment ceasing may explained the success obtained in postponing of diabetes onset. Vanadate treatment restored Glc6P level in liver and decreases hyperglycemia. **Abbreviations:** Glc6P (glucose-6-phosphate); Glc6Pase (glucose-6-phosphate hydrolase); CP:GlcPTase (CarbamyI phosphate: glucose phosphotransferase activity).

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SIMILAR CONTRIBUTION OF GLUCONEOGENESIS TO FASTING GLUCOSE PRODUCTION AT LOW AND HIGH GLUCOSE CONCENTRATIONS IN PATIENTS WITH TYPE 1 DIABETES MELLITUS

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Background and Aims: Elevated rates of gluconeogenesis are believed to play an important role in the pathophysiology of hyperglycemia in patients with diabetes mellitus. We therefore assessed glucose production (GP) and the contribution of gluconeogenesis (GNG) in fourteen type 1 diabetic patients, at a low glucose level (LO, $n=7$) and a high glucose level (HI, $n=7$).

Materials and Methods: Subcutaneous insulin was withdrawn 24 hours prior to each study, and the subjects were fasted overnight while their plasma glucose concentrations were maintained at the LO or HI levels by intravenous insulin infusion. GP was determined in the postabsorptive state by infusions of 6,6- 2 H $_2$ -glucose, and GNG was estimated by the deuterated water method. Glycogenolysis was calculated as the difference between rates of glucose production and gluconeogenesis.

Results: Fasting plasma glucose levels were higher in the HI than in the LO group (11.1 ± 0.7 vs. 6.2 ± 0.2 mM, $p < 0.001$) due to lower plasma insulin levels (39 ± 5 vs. 66 ± 8 pM, $p < 0.01$). In both groups, glucosuria was less than 2 mg/m 2 /min. Plasma free fatty acids (FFA) were similar in the HI and LO groups (0.5 ± 0.05 vs. 0.5 ± 0.05 mM, NS), whereas plasma glucagon levels were higher in the LO group (7.9 ± 0.4 vs. 5.1 ± 0.6 pM, $p < 0.02$). Despite these differences, rates of GP in the HI and LO group were equal (81 ± 4.2 vs. 81 ± 3.8 mg/m 2 /min, NS). Furthermore, % GNG (55 ± 3 vs. $57 \pm 2\%$, NS) as well as rates of gluconeogenesis (44 ± 3.4 vs. 44 ± 2.2 mg/m 2 /min, NS) and glycogenolysis (37 ± 3.5 vs. 37 ± 3.2 mg/m 2 /min, NS) were similar in the HI and LO group, respectively. In both groups, rates of GP, GNG and glycogenolysis were independent of the plasma levels of glucose, insulin, glucagon and FFA.

Conclusion: In type 1 diabetic patients without significant glucosuria, fasting rates of glucose production and the contributions of gluconeogenesis, are similar at low and high glucose levels.

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REGULATION OF GLUCOSE PHOSPHORYLATION BY TRANSLOCATION OF GLUCOKINASE BETWEEN NUCLEUS AND CYTOPLASM IN HEPATOCYTES

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Aims: The implication of glucokinase (GK) in the regulation of hepatic glucose metabolism still remains elusive. We studied the role of the translocation of hepatic GK between the nucleus and the cytoplasm in the regulation of glucose phosphorylation. **Materials and Methods:** Distribution of nuclear and cytoplasmic GK in cultured rat hepatocytes or rat liver sections was quantitatively estimated by analysis of confocal images of cells or sections stained by the immunofluorescence technique. Intracellular glucose phosphorylation was measured from the rate of release of 3 H $_2$ O from D-[2- 3 H]glucose by cultured hepatocytes. **Results:** Both the translocation of GK from the nucleus to the cytoplasm and the rate of glucose phosphorylation in cultured hepatocytes were increased as the medium glucose concentration was increased from 5 mM up to 40 mM. There was a good correlation between the increase in cytoplasmic GK induced by fructose, which sugar is known to stimulate glucose phosphorylation, and that in the glucose phosphorylation rate induced by fructose. A linear relationship between the cytoplasmic GK activity and the rate of glucose phosphorylation over various glucose concentrations was also observed either in the absence or presence of fructose. Oral administration of 2.5 ml of 20% glucose, 2.5% fructose, or 20% glucose plus 2.5% fructose to 24-h fasted rats induced translocation of GK from the nucleus to the cytoplasm in hepatic parenchymal cells by 30 min after sugar loading. **Conclusions:** The data indicate that hepatic glucose phosphorylation depends on GK activity in the cytoplasmic compartment and is regulated by the translocation of GK.

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TRANSCRIPTIONAL REGULATION OF THE GLUCOSE-6 PHOSPHATASE GENE IN STABLY-TRANSFECTED CaCo-2 CELLS DURING ENTEROCYTE DIFFERENTIATION.

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Background and Aims: Glucose-6-phosphatase (G6Pase) is a key enzyme in glucose homeostasis, which catalyzes the terminal step of gluconeogenesis. The expression of the G6Pase gene is restricted to the liver, the kidney and the small intestine, and is increased during diabetes and fasting in all three tissues. In order to study the transcriptional regulation of the G6Pase gene in enterocytes, we have taken advantage of the human colon adenocarcinoma CaCo-2 cell line, which is able to differentiate into enterocyte-like cells after several days of culture at post-confluence. At this time only, they express the endogenous G6Pase gene.

Materials and Methods: The -1640/+60 bp region of the G6Pase gene was cloned upstream of a luciferase reporter gene containing a selectable marker conferring resistance to puromycin. G6Pase promoter constructs were transfected in undifferentiated CaCo-2 cells. The stably-transfected clones containing the G6Pase promoter were selected using puromycin and called CaCo-2/G6Pase-prom. cells. Promoter activity was assessed by luciferase activity corrected by proteins concentration.

Results: In CaCo-2/G6Pase-prom cells, G6Pase promoter activity was low until the time of confluence and increased 2-fold after 1 week and up to 4 weeks after post-confluence. Moreover, at this time, the G6Pase promoter activity was significantly stimulated by 1.4 and 2 fold in the presence (for 48h) of 10-6M and 10-5M forskolin, respectively.

Conclusions: These results indicate that the G6Pase gene expression is induced by forskolin in enterocyte, like in hepatocyte. The CaCo-2/G6Pase-prom. cell line will be a useful tool to study the regulation of the G6Pase gene transcription in enterocyte.

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Elucidation of the molecular interaction between glucokinase and glucokinase regulatory protein

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Background and Aims: The low-affinity glucose phosphorylating enzyme glucokinase (GK) regulates glycolytic flux at millimolar glucose concentrations in liver and pancreatic beta cells. Hepatic GK activity is modulated on the posttranslational level through an interaction with a regulatory protein (GRP). Most recent studies demonstrate, that the GRP is responsible for the intracellular translocation of GK. We could recently identify an asparagine-leucine consensus motif within the GK protein by phage display library screenings which confer the binding to GRP. It was the aim of this study to characterize the molecular mechanisms underlying the interaction between GK and GRP. **Materials and Methods:** Site-directed mutagenesis of selected amino acids in the GRP and GK protein was performed by the Altered Sites II in vitro Mutagenesis System. Protein interactions of wild type and mutant proteins were characterized by the MATCHMAKER GAL4 Two-Hybrid System 2 and verified by growth selection and quantitative chemiluminescence β -galactosidase assays. GK enzyme activities were measured by a photometric assay. **Results:** An asparagine-leucine consensus motif of GK for interaction with GRP could be localized in three areas of the protein, Leu-309/Asn-313 (A), Asn-350/Leu-355 (B) and Leu58/Asn-204 (C). Binding motif A is part of a proposed nuclear export signal whereas motif C is located within the substrate binding site of GK. Only the double mutation of the amino acids Leu-58/Asn-204 inside the substrate binding site of the GK protein resulted in a complete loss of the interaction with GRP. In addition, we found that also the single mutations of Leu-58 and Asn-204 resulted in a complete loss of binding to GRP. In enzyme activity studies these mutations showed a significant loss of GK affinity for the substrate glucose. In contrast, the double mutation Asn-350/Leu-355 and the single mutation Leu-355 retained a significant interaction of GK/GRP in the range of 50 % of the wild type GK protein. **Conclusion:** The amino acids Leu-58 and Asn-204 of the GK protein play the most important role for the interaction between GK and GRP. As Asn-204 is localized in the substrate binding site the interaction of GK with GRP affects the catalytic function.

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PROTEIN EXPRESSION LEVELS OF THE HEPATIC GLUCOKINASE REGULATORY PROTEIN (GLKRP) VARY IN A RANGE OF ANIMAL MODELS OF TYPE 2 DIABETES.

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Aims: We used a new monospecific antisera to the glucokinase regulatory protein (GLKRP) to determine expression levels in differing animal models of diabetes. These included ob/ob and db/db mice and Zucker and Zucker fatty Diabetic (ZDF) rats. In addition we tested high and low glucokinase (GLK) expressing mice C3H and C58 for GLKRP expression. **Materials and Methods:** Recombinant rat GLKRP was used to immunise 3 New Zealand White rabbits. Each animal received 4 doses over a 20 week period. Liver samples were run on 8-16% Tris-Glycine gels. After transfer to nitro-cellulose membrane and blocking of the membrane, GLKRP was detected using the purified antisera. The protein bands were visualised using a Western Breeze kit (InVitrogen). GLK antisera was from SantaCruz. **Results:** In mice the highest expression of GLKRP was in ob/ob and db/db. However db/db mice expressed significantly less GLK than either ob/ob or control mice. C3H expressed significantly more GLK than C58, as was expected, and a similar pattern was observed for GLKRP. In the rat models both the Zucker and the ZDF expressed significantly more GLKRP than controls however both contain marginally less GLK than controls. **Conclusions:** We conclude from these results that careful consideration should be given to the selection of the appropriate animal model, based on expression of GLK and GLKRP when testing agents which affect the early steps of hepatic carbohydrate metabolism.

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Pancreatic Glucose and Methionine Uptake in Vivo in Healthy Men and Men with Newly Diagnosed Type 1 Diabetes.

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Background and Aims: Because activated lymphocytes utilize up to 50 times more glucose than resting cells, we evaluated possibilities to visualize insulinitis in vivo with positron emission tomography (PET) using 18F-2-fluoro-2-deoxy-D-glucose (FDG) and 11C-methionine as uptake markers.

Materials and Methods: Nine male patients with newly (3 ± 2 months) diagnosed multiple autoantibody positive type 1 diabetes and 6 age-matched healthy control males were studied during euglycemia after an overnight fast. For definition of the regions of interest (ROIs) in the main body of pancreas, the organ was first localized with MRI and with 11C-methionine, which showed high uptake to the exocrine pancreas and accurately visualized the pancreas also in the PET. The accumulated FDG and 11C-methionine data were analyzed graphically.

Results: Fractional 11C-methionine uptake, which especially in the body of the pancreas was intense, was slightly lower in the pancreas of the patients with diabetes than the controls ($K_i = 0.13 \pm 0.05$ vs. 0.19 ± 0.09 , $p=0.08$). Fractional FDG uptake was not significantly different between the patients and the controls (0.0033 ± 0.0010 vs. 0.0026 ± 0.0013 , $p=0.15$), but plasma glucose-corrected FDG uptake was higher in the patients with diabetes than in the controls (2.0 ± 0.7 vs. 1.3 ± 0.6 mmol/100 g of tissue/min, $p=0.03$). The ratio between glucose and 11C-methionine uptake in the pancreas was twice as high in the patients with type 1 diabetes than in the controls ($p<0.05$).

Conclusions: We conclude that adult men with type 1 diabetes show slightly lower pancreatic methionine uptake, but glucose uptake to the pancreas is enhanced. The data suggest that proper cellular or membrane markers of T cell activation may produce signal intensities in PET that allow in vivo visualization of insulinitis at least when the islet infiltrating cells remain functionally active.

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Molecular Insulin Resistance

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Skeletal muscle cells from insulin resistant (non-diabetic) individuals are susceptible to insulin desensitization by fatty acids

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Background and Aims: We recently demonstrated that insulin sensitivity in cultured skeletal muscle cells was not different between insulin sensitive and insulin resistant subjects (Diabetes 2000; 49, 992-998). One possible interpretation of these findings was that insulin action or signalling in these individuals was not primarily defective. In the present studies therefore, we tested the hypothesis that incubation of isolated skeletal muscle cells with fatty acids has an effect on insulin action which differs between insulin sensitive and insulin resistant subjects.

Materials and Methods: 5-day fused myotubes from 6 lean insulin-resistant (IR) and 6 carefully matched insulin-sensitive (IS) subjects (metabolic clearance rates of glucose determined by euglycemic-hyperinsulinemic clamp: 4.5 ± 0.03 vs. 13.8 ± 1.95 ml kg^{-1} min $^{-1}$; $p < 0.001$) were incubated in the absence or presence of palmitate, 2-bromo-palmitate and linoleate for 20h.

Results: Insulin-stimulated (100 nM) glycogen synthesis decreased by $46 \pm 1\%$ in cells from IR subjects but remained unchanged in cells from IS subjects (p vs IR = 0.02). PI-3 kinase activity decreased by $36 \pm 2\%$ in cells from IR subjects, but was not different in cells from IS subjects (p vs IR = 0.07; $n = 4$ pairs). PI 3-kinase p85 protein expression was unaltered. No significant differences were found for glycogen synthesis and PI 3-kinase activity after linoleate and 2-bromo-palmitate treatment. Furthermore, insulin activation of PKB/Akt, GSK-3, and the respective protein expression were not different after palmitate, linoleate or 2-bromo-palmitate treatment ($n = 4$ pairs).

Conclusions: Palmitate but not 2-bromo-palmitate incubation impaired insulin action in cells from IR but not from IS subjects. Our data thus provide preliminary evidence that insulin resistance of skeletal muscle does not necessarily involve primary defects in insulin action but could represent susceptibility to the desensitizing effect of fatty acids (and possibly other environmental factors). This effect appears to be coupled to fatty acid metabolism.

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DIFFERENTIAL EFFECTS OF FATTY ACIDS ON RINm5F CELLS DEPEND ON SATURATION AND CHAIN LENGTH

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Background and Aims: Saturated fatty acids, such as palmitic acid, have been shown to induce nitric oxide synthase and apoptotic cell death in some tissues. In this study, we determined whether saturation and chain length of fatty acids influences RINm5F cell function, survival and expression of inducible nitric oxide synthase (iNOS).

Methods: RINm5F cells were exposed for 12-72h to six fatty acids which were: saturated - palmitic acid C16:0 (PA) or stearic acid C18:0 (SA), monounsaturated - palmitoleic acid C16:1 (POA) or oleic acid C18:1 (OA), and polyunsaturated - linoleic acid C18:2 (LA) or docosahexaenoic acid C22:6 (DHA). Cell viability and insulin secretion were measured. iNOS protein was quantified by Western blotting; Hoechst-propidium iodide staining was used to determine apoptosis and necrosis. **Results:** Exposure only to saturated fatty acids (50-200µM) decreased RINm5F cell survival - untreated cells 31.3 ± 3.3 ; (PA 50µM) 8.7 ± 2.0 $P<0.001$ and (SA 50µM) 10.2 ± 1.8 mg cell protein $P<0.001$. Apoptosis rose from 0.4 % to 9.1% (PA) and 8.1% (SA) respectively. Western blot analysis confirmed that iNOS protein was not expressed in response to PA, POA, SA, or DHA treatment for 24h. However, interleukin-1β-induced iNOS expression was down-regulated and nitric oxide (nitrite) formation decreased by DHA (150mM) (117 vs. 52 integrated density units). DHA also normalised the IL-1 effect on insulin secretion.

Conclusions: The deleterious effects of fatty acids on RINm5F cells are specific to saturated forms. Saturated fatty acid cytotoxicity does not involve induction of iNOS but, unexpectedly, the polyunsaturated C22:6 fatty acid docosahexaenoic acid was found to down regulate the expression of iNOS.

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Tumor necrosis factor- α inhibits insulin-stimulated glucose uptake in humans
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Background and aims: In patients with insulin resistance, obesity, and type 2 diabetes, adipose tissue overexpress tumor necrosis factor- α (TNF), and circulating TNF concentrations are elevated. Furthermore, TNF has been shown to inhibit insulin-stimulated glucose uptake in animal models and in vitro, and may thus be a mediator of insulin resistance. The aim of this study was to examine whether TNF acutely inhibits insulin-stimulated glucose uptake in lean, healthy humans.

Materials and methods: Male, lean, healthy volunteers were examined at two different occasions separated by >3 weeks, starting in the morning in the fasting state. The perfused forearm method was used by infusing drugs into the brachial artery and measuring forearm blood flow by venous occlusion plethysmography. Glucose uptake was measured as the product between the arterial-venous difference of plasma glucose and forearm blood flow and is expressed below as micromoles per 100 ml tissue per minute. Insulin 0.05 mU/min was infused for 20 minutes; we have previously shown that this increases serum insulin in the perfused arm by a factor ~ 10 with a marginal increase in systemic serum insulin. After 60 minutes with saline infusion only, human recombinant TNF was infused for 10 minutes. Subsequently, TNF infusion was continued with co-infusion of either insulin (day T+1, n=12) or vehicle (day T, n=9) for 20 minutes.

Results: Insulin stimulation increased glucose uptake 98% (from 0.8 ± 0.1 to 1.6 ± 0.2 , pooled results from both days, $p=0.001$). As expected, glucose uptake at baseline before TNF infusion (1.2 ± 0.1) was higher than at baseline before the primary insulin stimulation (0.8 ± 0.1), although not significantly so ($p=0.09$). TNF infusion alone raised plasma TNF from 1.4 ± 0.5 to 13.4 ± 3.6 in the perfused arm and to 6.5 ± 1.4 ng/l in the contralateral arm, but did not significantly change glucose uptake on either day (from 1.2 ± 0.2 to 1.0 ± 0.1 , $p=0.3$). Repeated insulin stimulation during co-infusion with TNF (day T+1) resulted in an insignificant 36% increase in glucose uptake (from 0.9 ± 0.2 to 1.2 ± 0.1 , $p=0.18$). After co-infusion of TNF with vehicle (day T), glucose uptake decreased insignificantly (from 1.2 ± 0.3 to 0.9 ± 0.2 , $p=0.09$).

Conclusions: To our knowledge, this is the first direct demonstration that TNF inhibits insulin-stimulated glucose uptake in humans. The results support the concept that TNF may mediate insulin resistance in individuals with obesity and type 2 diabetes.

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LIVER FAT CORRELATES WITH FEATURES OF INSULIN RESISTANCE INDEPENDENT OF OBESITY IN PREVIOUS GESTATIONAL DIABETES

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Background and aims: Extra-adipose tissue lipogenesis in muscle has been associated with insulin resistance of glucose uptake. We determined whether ectopic lipogenesis in the liver is associated with insulin resistance of hepatic glucose production in previously gestational diabetic obese women. **Material and methods:** We recruited 26 obese (age 37 ± 1 yrs., BMI $28-35$ kg/m²) previously gestational diabetic women. Liver fat content was determined by proton spectroscopy, insulin sensitivity by using the euglycemic insulin clamp technique, glucose tolerance by OGTT and large artery stiffness, a recently described feature of the insulin resistance syndrome, by measuring the augmentation index with pulse wave analysis.

Results: Within this group of obese women, the % liver fat was not correlated with either BMI ($r=0.26$, NS) or the waist to hip ratio ($r=0.29$, NS). The % liver fat correlated, however, with several features of insulin resistance: fasting serum insulin ($r=0.53$, $p=0.006$), the 2 hr plasma glucose concentration ($r=0.49$, $p=0.01$), serum triglycerides ($r=0.50$, $p=0.01$) and whole body insulin sensitivity ($r=-0.42$, $p=0.03$). Liver fat content also correlated with large artery stiffness ($r=0.61$ for the augmentation index, $p=0.001$). **Conclusions:** Clinical and biochemical features of insulin resistance correlate with liver fat content independent of obesity amongst obese women with previous gestational diabetes. These data demonstrate that some women have a stronger tendency to deposit fat outside adipose tissue ('ectopic lipogenesis') than others. These women have a more adverse cardiovascular risk profile than those who can keep fat where it belongs.

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PRE-HYPERGLYCAEMIC EFFECTS OF A HIGH FAT DIET IN THE VERVET MONKEY

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Background and Aims: The transition from rural to urban life of the South African black populations is associated with an increased incidence of obesity and type 2 diabetes. Evidence suggests that this is related, in part, to increased fat consumption, possibly exacerbated by *in utero* programming for the limited diet available in rural areas. The aim of this study is to emulate the dietary changes associated with this transition in the Vervet monkey and monitor the long-term effects on weight, glucose clearance and plasma levels of insulin, pro-insulin, glucagon, free fatty acids (FFAs), leptin and cholesterol. **Materials and Methods:** A group of monkeys (n=5) is being maintained on the same quantity of a high fat (43%) diet (HFD) as fed to a control group on a maintenance (20%) diet. Weight, GTTs and plasma analyses were recorded at regular intervals. **Results:** A rapid initial effect was observed, at 17 days, of reduced insulin levels and glucose clearance rates and raised levels of glucagon, FFAs, plasma and LDL cholesterol and leptin. Insulin, glucose clearance rate and leptin levels returned to normal after four to five months but then became progressively unstable in two of the monkeys. Fasting pro-insulin: insulin ratios progressively increased after 7 months of diet in three monkeys. The highest weight increases of 52% and 59% were in the two monkeys most compromised by the diet. Despite all of these changes, fasting glucose levels remained within the normal range after two years of a HFD although fasting HbA_{1c} values in all of the monkeys steadily increased after 19-21 months of the HFD. **Conclusion:** A HFD appeared to have a rapid and sometimes sustained effect on all of the parameters measured in this study. Not every monkey exhibited the same combination of effects and the individuals with the largest weight gain appeared to be most compromised by the diet. Glucose levels remain in the normal range after 2 yrs of diet but HbA_{1c} values were increasing above the normal range in all five monkeys after 18 months of the diet.

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Muscle Mass Heavy Chain (MHC) Gene Transcripts in Type 2 Diabetes

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Background and Aims: Association of reduced skeletal muscle fiber type I and increased type IIb fibers have been reported to occur in people with type 2 diabetes and their non-diabetic first-degree relative with insulin resistance. It is unclear whether glycemic control causes these changes or the fiber types cause insulin resistance. Muscle fiber types are determined by the relative expression of MHC isoforms. We investigated the role that glycemic control and plasma insulin levels play in the MHC isoforms gene expression.

Materials and Methods: We determined MHC isoform gene transcript levels in vastus lateralis needle biopsy samples, using a real-time QPCR technique. We studied 7 type 2 diabetic patients (BMI=30.9 \pm 0.4 kg/m²) once after 2 weeks off treatment (D2-) and another time following 11 days of intensive insulin treatment (D2+) and in control subjects matched for age, gender and weight (C) (BMI = 30.2 \pm 0.4).

Results: Plasma glucose (D2- = 10.5 ± 0.88 mmol/l, D2+ = 4.9 ± 0.21 mmol/l, C = 4.8 ± 0.12 mmol/l) and insulin (D2- = 27.6 ± 4.22 pmol/l, D2+ = 70.3 ± 10.72 pmol/l, C = 40.8 ± 12.68 pmol/l) levels, as well as their insulin sensitivity (D2- = 3.21 ± 1.4 , D2+ = 3.16 ± 0.7 , C = 5.28 ± 1.2), were different among the groups ($P<0.01$). MHC I was lower in D2- (28.0 ± 6.7 AU) than C (55.3 ± 12.7) ($P<0.04$) and insulin treatment (D2+) (76.7 ± 17.0) abolished these differences. MHC IIa was higher in D2+ (3.95 ± 0.8 AU) than in D2- (2.3 ± 0.5) ($P<0.04$). MHC IIx was higher in D2+ (5.8 ± 0.4 AU) than D2- (2.3 ± 0.5) ($P<0.07$). MHC isoform gene transcript levels are varied based on circulating insulin levels and glycemic control.

Conclusions: The altered MHC isoform gene transcription in skeletal muscle in people with type 2 diabetes is related to their treatment status and is unlikely to be the basis of their muscle insulin resistance.

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Hexosamine biosynthesis pathway intermediates and protein kinase C-isoforms in muscle and fat tissue of Zucker diabetic fatty rats

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Background and Aims: Many studies suggest that insulin resistance develops and/or is maintained by an increased flux of glucose through the hexosamine biosynthesis pathway. In addition, it is suggested that this pathway attenuates insulin-stimulated glucose uptake by activating protein kinase C (PKC). In general, it is believed that PKC plays a pivotal role in the cellular effects induced with hyperglycemia. We investigated 1) by HPLC analysis the concentration of the major hexosamine metabolites and 2) by Western blotting the expression levels of PKC-isoforms in Zucker Diabetic Fatty (ZDF) rats, an animal model for type 2 diabetes mellitus.

Results: At the age of 6 weeks these rats were normoglycemic. While control rats remained normoglycemic, the ZDF rats became clearly hyperglycemic with blood glucose concentrations of approximately 25 mM at 12 weeks of age and older. First, the amount of uridine diphosphate-N-acetyl-glucosamine (UDP-GlcNAc) and uridine diphosphate-N-acetyl-galactosamine (UDP-GalNAc), the major end-products of the hexosamine biosynthesis pathway, was determined in muscle tissue of ZDF rats at 6, 12, 18 and 24 weeks of age. Despite the chronic hyperglycemia, when ZDF rats were 12 weeks of age and older, no increase in the amount of the two UDP-linked hexosamines was detected. Moreover, the concentration of UDP-GlcNAc and UDP-GalNAc did not differ from (normoglycemic) control rats. Finally, maintaining the blood glucose concentration at 7 mM of ZDF rats by administration of phlorizin did not affect the levels of UDP-GlcNAc and UDP-GalNAc. Second, we assessed which PKC-isoforms are expressed in muscle and fat tissue, and whether their levels were affected in ZDF rats. In muscle and fat tissues from (normoglycemic) 6 and (hyperglycemic) 24 weeks old ZDF rats, PKC α , δ , ϵ , ζ and η are expressed, but not PKC β and γ . A similar pattern was observed in tissues from control rats of identical age. By comparison of ZDF and control rats at 24 weeks of age we observed no difference in expression levels of any of the PKC-isoforms in both muscle and fat tissue.

Conclusions: In summary, our data do not support a prominent role for the hexosamine synthesis pathway and PKC in the development or maintenance of the insulin-resistant state of muscle and fat tissue in the ZDF rats.

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HYPERGLYCEMIA-INDUCED INSULIN RESISTANCE IS NOT CAUSED BY OVERACTIVITY OF THE HEXOSAMINE PATHWAY IN TYPE 2 DIABETES

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Background and Aims: Animal studies suggest that overactivity of the hexosamine pathway, resulting in increased UDP-hexosamine levels, is a major mechanism by which hyperglycemia causes insulin resistance. This study was performed to test this hypothesis in diabetic patients. **Materials and Methods:** Protocol A: 8 obese, insulin resistant patients with uncontrolled type 2 diabetes despite insulin treatment (BMI 38 \pm 5.8 kg m $^{-2}$, HbA1c 12.0 \pm 1.7 %, insulin dose 1.92 \pm 0.65 U/kg/day) were treated with insulin i.v. for 28 \pm 6 days aimed at strict euglycemia. Before and after treatment, insulin sensitivity was measured using a hyperinsulinemic euglycemic clamp, and a muscle biopsy (m. vastus lateralis) was taken for measurement of metabolites of the hexosamine route. Protocol B: In 8 diabetic (type 2) and 46 nondiabetic patients, who underwent hip replacement surgery, muscle tissue (m. gluteus maximus) biopsies were obtained. **Results:** Protocol A: After euglycemia, hyperglycemia-induced insulin resistance reversed, as demonstrated by an increase in whole body glucose uptake during the clamp from 12.7 \pm 5.6 to 22.3 \pm 8.8 micromol kg $^{-1}$ min $^{-1}$ and a decrease in insulin requirement from 176 \pm 88 to 115 \pm 70 U/day, while metabolic control improved (HbA1c levels decreased to 8.6 \pm 1.1%). UDP-glucose (substrate for glycogen synthesis) was undetectable before and after treatment. UDP-hexosamine levels (end products of the hexosamine pathway) increased significantly from 13.3 \pm 1.8 to 18.1 \pm 4.0 nmol g tissue $^{-1}$ (P<0.01). Protocol B: Mean UDP-glucose concentration was lower (5.6 \pm 3.4 nmol g tissue $^{-1}$) in the diabetes group compared to the nondiabetes group (15.8 \pm 8.4 nmol g tissue $^{-1}$ (P<0.01)), and UDP-hexosamine concentrations were not significantly different. **Conclusions:** After amelioration of hyperglycemia-induced insulin resistance, end products of the hexosamine pathway increased in skeletal muscle of patients with type 2 DM (protocol A). Hexosamine pathway metabolites were similar between diabetic and non-diabetic patients (protocol B). These results do not support the hypothesis that hexosamine metabolic endproducts are involved in the pathogenesis of glucose-induced insulin resistance in patients with type 2 DM.

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Simultaneous treatment of isolated skeletal muscle with interleukin 6 and tumour necrosis factor decreases insulin stimulated glycogen synthesis

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Background and Aims: A primary defect of insulin resistant skeletal muscle is decreased insulin-mediated glycogen synthesis but the mechanisms responsible for this decrease are not completely understood. Insulin resistance is also present with inflammation and infection. Recently, small increases in concentrations of cytokines such as tumour necrosis factor (TNF) and interleukin 6 (IL6) have been observed in insulin resistant conditions such as type II diabetes and obesity. It has been reported that TNF does not affect glucose metabolism in isolated skeletal muscle but the effect of IL6 on insulin action in isolated skeletal muscle has not been investigated. The aim of this study was to determine whether IL6 by itself or in combination with TNF has any effect on insulin action in skeletal muscle. **Materials and Methods:** Soleus muscle strips isolated from male Wistar rats were incubated for four hours in vitro in the absence of cytokines (control), in the presence of IL6 (100, 200, 500pg/mL) and/or TNF (10ng/mL) in Krebs-Ringer bicarbonate buffer containing 5.5mM glucose in an atmosphere of 95%O $_2$: 5%CO $_2$. Muscles were then incubated in similar media that also contained insulin (10 or 1000 μ U/mL) and tracer quantities of 14C-glucose and 3H-2-deoxyglucose for one hour. Insulin-mediated glucose transport/phosphorylation and glycogen synthesis were then determined. All results are expressed as mean \pm SEM in μ mol/h/g wet wt. **Results:** Insulin (1000 μ U/mL) caused a significant increase above basal in the rates of glucose transport/phosphorylation (2 fold; p<0.001) and glycogen synthesis (6 fold; p<0.001). There was no effect of cytokine treatment on the insulin stimulated rate of glucose transport/phosphorylation (control 3.48 \pm 0.17; TNF 3.43 \pm 0.22; IL6 3.56 \pm 0.17; combination 3.24 \pm 0.11). IL-6 alone (500pg/mL) or TNF alone did not affect insulin stimulated glycogen synthesis. However, the combination of IL6 (500pg/mL) and TNF (10ng/mL) significantly decreased insulin stimulated glycogen synthesis (control 2.50 \pm 0.32 of combination: 1.69 \pm 0.14 p<0.01). In the presence of TNF, IL-6 decreased glycogen synthesis in a concentration dependent manner (0pg/mL: 2.32 \pm 0.21; 100pg/mL: 2.47 \pm 0.04; 200pg/mL: 1.95 \pm 0.20; 500pg/mL: 1.71 \pm 0.15 p<0.05). **Conclusions:** This study shows that when combined, TNF and IL6 impair insulin stimulated glycogen synthesis. TNF has been reported to upregulate IL6 receptors in myocytes. The latter may be the basis for the combined effect on glycogen synthesis and provide a mechanism by which these cytokines have a direct role in altering skeletal muscle insulin action in vivo.

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INCREASED TUMOR NECROSIS FACTOR- α SYSTEM ACTIVITY IN OBESE SUBJECTS WITH IMPAIRED GLUCOSE TOLERANCE.

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Background and aims: Tumor necrosis factor- α (TNF α) may be one of the factors linking obesity with type 2 diabetes. However, data about TNF α system activation in impaired glucose tolerance (IGT) and diabetes are controversial. The aim of the present study was to evaluate plasma levels of TNF α and soluble fractions of TNF α receptors (sTNFR1 and sTNFR2) in obese subjects with normal glucose tolerance (NGT) and IGT and to examine whether those levels are related to insulin sensitivity. **Materials and methods:** We examined 11 obese subjects with IGT and 16 with NGT. 13 healthy lean subjects served as a control group. In all the subjects anthropometrical and biochemical parameters and plasma levels of TNF α , sTNFR1 and sTNFR2 were measured. Hyperinsulinemic euglycemic clamp (insulin infusion: 50 mU x kg $^{-1}$ x hour $^{-1}$) was performed to determine insulin sensitivity. **Results:** Anthropometrical measurements did not differ between the two groups of the obese subjects. Plasma TNF α and sTNFR2 were markedly higher in both groups of obese subjects in comparison to controls and in the IGT vs NGT group (p<0.05 in all cases). Plasma sTNFR1 did not differ between the studied groups. IGT subjects were also more insulin resistant in comparison to NGT group (p<0.001) and controls (p<0.0005) and the NGT subjects were more insulin resistant than controls (p<0.01). Plasma sTNFR2 were markedly related to insulin sensitivity when all the study population was analyzed (r=-0.64; p<0.001) and also in the IGT (r=-0.81; p<0.005) and NGT (r=-0.52; p<0.05) groups. Those relationships remained significant after adjustment for BMI, WHR, percent of body fat and plasma glucose. **Conclusions:** Our data suggest that TNF α system contributes to the development of insulin resistance in glucose intolerant subjects.

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PLASMA TUMOR NECROSIS FACTOR-ALPHA LEVELS AND INSULIN RESISTANCE IN NONDIABETIC HYPERTENSIVE SUBJECTSB. DEMIRBAS, S. GULER, B. CAKIR, I. SAHIN, C. CULHA, Y. ARAL
Ankara Education and Research Hospital, Department of Endocrinology, Ankara, TURKEY.**Background and Aims:** Recent studies have shown that tumor necrosis factor-alpha (TNF-alpha) is associated with insulin resistance. However, whether TNF-alpha is related to insulin resistance in hypertensive subjects is still controversial. The aim of this study was to determine the status of TNF-alpha and insulin resistance in hypertension.**Materials and Methods:** Newly diagnosed nondiabetic 17 essentially hypertensive (6 men, 11 women) patients, and 11 control healthy subjects (5 men, 6 women) are involved in the study. Body mass index (BMI), waist/hip ratio (WHR), insulin, subcutaneous fat tissue thickness, fasting blood glucose, cholesterol, triglyceride, and TNF-alpha levels were measured. Insulin resistance is assessed according to homeostasis model of assessment (HOMA-IR).**Results:** Serum insulin (8.4 ± 2.7 vs. 6.1 ± 1.4 mIU/ml; $p < 0.01$), triglyceride (245.0 ± 39.9 vs. 193.0 ± 22.8 mg/dl; $p < 0.01$), and TNF-alpha (4.1 ± 1.4 vs. 1.7 ± 1.7 pg/ml; $p < 0.001$) levels, and HOMA-IR (2.0 ± 0.8 vs. 1.3 ± 0.3 ; $p < 0.001$) were significantly higher in the hypertensive patients compared to the normotensive control group. There were positive correlations between TNF-alpha levels and body mass index ($r = 0.5$, $p < 0.01$), subcutaneous fat tissue thickness ($r = 0.56$, $p = 0.002$), cholesterol ($r = 0.51$, $p = 0.006$) and triglyceride ($r = 0.57$, $p = 0.002$) levels, and positive correlations between HOMA and subcutaneous fat tissue thickness ($r = 0.43$, $p < 0.03$) in the whole study group. However there were no correlation of either TNF-alpha or HOMA-IR with any of the study parameters when hypertensive and normotensive groups are analyzed separately.**Conclusions:** Our data revealed that hypertensive patients have insulin resistance and higher TNF-alpha levels, but there is no relation between TNF-alpha levels and insulin resistance.

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Reduced glucose uptake in skeletal muscle of partially hexokinase II deficient mice.

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Background and Aims: Type 2 diabetes is characterized by decreased rates of insulin-stimulated glucose uptake and utilization, reduced hexokinase II (HKII) mRNA and enzyme production, and low basal levels of glucose-6-phosphate in insulin-sensitive skeletal muscle and adipose tissues where HKII is primarily expressed to catalyze the phosphorylation of glucose to glucose-6-phosphate. We have previously generated HKII knock-out mice and shown that, although 100% HKII deficiency (HKII^{-/-}) leads to early embryonic lethality, 50% HKII deficiency (HKII^{+/-}) does not impair glucose tolerance of anesthetized mice, even if they were challenged with a high-fat diet. In this study, we investigated the effect of reduced HKII enzyme activity on tissue-specific glucose uptake.**Materials and Methods:** An intraperitoneal glucose tolerance test (2mg/g glucose) with non-metabolizable radioactive tracer (0.27μCi/g 2-deoxy-D-[1-³H]glucose (2DG)) was performed on female wild type and HKII^{+/-} mice (N=6+6) at 15 wk of age without anesthesia. For the analysis of tissue specific glucose uptake (Gupt; μmol/min/mg protein), blood samples, collected at 0, 15, 30, 60, 90, and 120min, were analyzed for plasma glucose and mean glucose specific activity (mGSA), and tissues, excised from mice killed at 120min, were analyzed for the accumulation of 2DG-6-phosphate (2DGP). Glucose uptake for each tissue was calculated by dividing the 2DGP content with mGSA, and normalized for brain Gupt.**Results:** In HKII^{+/-} mice, the accumulation of 2DGP was reduced by 40% in quadriceps ($p = 0.012$). In keeping with the phosphorylation defect, serum glucose concentration declined from 15 min to 120 min significantly slower in HKII^{+/-} mice than wild-type mice (69 ± 23 vs. 105 ± 28 μmol/l/min, $p < 0.05$).**Conclusions:** In this study, we show that the 50% HKII deficiency leads to reduced glucose phosphorylation in skeletal muscle, indicating impaired glucose uptake, which in turn might result in a mild defect in glucose metabolism. Although the 50% HKII deficiency is not likely to lead to insulin resistance or type 2 diabetes, our data suggest that after a glucose challenge in awake, partially HKII deficient mice, glucose phosphorylation appears to be a rate-limiting step for glucose metabolism.

PS 40

Experimental Insulin Resistance

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Activation of Protein Kinase C delta by a distinct pathway mediates Tumor Necrosis Factor-alpha inhibition of Insulin Receptor signaling.

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Background and Aims: Tumor Necrosis Factor (TNF)-alpha, a multifunctional cytokine thought to be involved in the development of insulin resistance, has been shown to inhibit insulin receptor (IR) signaling. The mechanism of this effect is not clear. Studies from our laboratory have shown that certain protein kinase C (PKC) isoforms are involved in the mediation of insulin effects in skeletal muscle. The purpose of this study was to examine the possibility that TNF-alpha may affect IR signaling via effects on specific PKC isoforms.**Materials and Methods:** Experiments were done on primary cultures of skeletal muscle, age 5-6 days in culture, obtained from newborn mice.**Results:** TNF-alpha given 5-min before insulin nearly completely prevented tyrosine phosphorylation of both IR and IRS-1, and caused a delay in insulin-induced IR internalization. Both TNF-alpha and insulin tyrosine phosphorylated and activated PKC delta, but the effects of TNF-alpha and insulin together were less than those of either substance alone. Insulin caused specific association between IR and PKC delta, an effect prevented by TNF-alpha. To further investigate the role of PKC delta in TNF-alpha inhibition of IR stimulation, we studied the effects of PKC delta overexpression and blockade. Overexpression of PKC delta increased IR tyrosine phosphorylation and abrogated the inhibitory effects of TNF-alpha on IR tyrosine phosphorylation, whereas overexpression of kinase inactive PKC delta inhibited both basal and insulin-induced IR phosphorylation. The results indicate that tyrosine phosphorylation of PKC delta by insulin and TNF-alpha involve different pathways. This is supported by findings that inhibition of Src tyrosine kinase reduced tyrosine phosphorylation of IR, PKC delta, and IR-PKC delta association induced by insulin but not by TNF-alpha.**Conclusions:** PKC delta is important for insulin-induced IR tyrosine phosphorylation and the continuation of IR signaling. Prior activation of PKC delta by TNF-alpha interferes with the role of PKC delta in IR signaling. We conclude that tyrosine phosphorylation of PKC delta induced by TNF-alpha occurs on different sites from those in response to insulin. The identification of these sites is currently under investigation.

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INOSITOL PHOSPHOGLYCANS AND INSULIN SENSITIVITY OF ADIPOCYTES FROM TWO STRAINS OF RATS; RELATION TO OBESITY.

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Background and Aims: An insulin second messenger, inositol phosphoglycan A-type (IPG-A), regulates key enzymes of lipogenesis. Evidence exists for its involvement in NIDDM and obesity. Our observations that adipocytes from two normal strains of Wistar rat (suppliers: Harlan Olac (HO) or Charles River (CR)) exhibited markedly different rates of lipogenesis from glucose in response to insulin and IPG-A *in vitro*, and that the accumulation of visceral fat was also different, prompted this study of the biochemical profiles of these two nominally similar strains of Wistar rats in order to throw light on the regulation of obesity and visceral fat accumulation. **Materials and Methods:** Adipocytes from the two Wistar strains (130-140g weight) were used to measure the effect of insulin (1nM) or IPG-A on lipogenesis. Leptin release and blood levels were measured using a RIA kits. Results are mean±SEM of not less than 6 separate experiments. **Results:** Lipogenesis from [¹⁴C] glucose was stimulated +668±72% and +264±45% with insulin in adipocytes from CR and HO rats respectively; the equivalent values for IPG-A stimulation were +23±4% and +99±9%; the tissue content of IPG-A was 3.5 times higher in CR/HO rats, in line with the basal rate of lipogenesis being 2-fold higher in adipocytes from CR/HO. Enzymes of lipogenesis (ATP citrate lyase, malic enzyme, acetyl CoA carboxylase, fatty acid synthase) were all significantly higher (150-200%) in CR/HO; the rate of lipolysis CR/HO was +160% ($P < 0.05$). The respective fat pad weights were 0.491 ± 0.017 and 0.616 ± 0.029 ($P < 0.001$); DNA (mg/g fat pad) and blood glucose were not different. Other changes were the lower cAMP content of adipose tissue and the higher plasma leptin/insulin ratios in CR/HO rats. **Conclusions:** The apparent anomalous result of a higher rate of lipogenesis and response to insulin combined with a lower accumulation of fat pad lipid in CR group is interpreted as being due to a faster turnover of lipid, both synthesis and lipolysis, which, combined with the known effects of leptin in raising fatty acid oxidation by muscle and depressing insulin secretion, could be central to the lower rate of accumulation of visceral fat.

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Oral Treatment with a Glycogen Synthase Kinase-3 Inhibitor Improves Glucose Tolerance and Skeletal Muscle Glucose Transport Activity in Zucker Diabetic Fatty Rats

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Background and Aims: Recent evidence has demonstrated a link between elevated glycogen synthase kinase-3 (GSK3) activity and insulin resistance in skeletal muscle of type 2 diabetic subjects, and we have shown that *in vitro* inhibition of GSK3 activity enhances insulin-mediated glucose transport activity in skeletal muscle of the markedly insulin-resistant Zucker Diabetic Fatty (ZDF) rat. In the present investigation, we have assessed the effects of acute oral treatment of ZDF rats with a novel small organic GSK3 inhibitor (CT98023) on glucose tolerance and skeletal muscle glucose transport activity.

Materials and Methods: Male ZDF rats (9-10 weeks old, ~330 g) were dosed twice by gavage over a 3.5-hour period with either vehicle (1% CMC/0.1% Tween) or 30 mg/kg CT98023. An oral glucose tolerance test (OGTT; 1 g/kg; 120 min) and assessment of insulin-stimulated glucose transport activity (2-deoxyglucose uptake; 1 mM) in isolated epitrochlearis and soleus muscles were then completed.

Results: The levels of CT98023 were enhanced in plasma and skeletal muscle by the treatment. The glucose and insulin responses during the OGTT were reduced by 45% and 75%, respectively (both $p < 0.05$), in the GSK3 inhibitor-treated animals. Thirty minutes after the final GSK3 inhibitor treatment of the ZDF rats, stimulation of glucose transport with either submaximally effective (100 μ U/ml) or maximally effective (5 mU/ml) insulin concentrations was significantly enhanced in isolated epitrochlearis (50% and 57%) and soleus (40% and 43%) muscles. Two hours after the final GSK3 inhibitor treatment, maximal insulin-mediated glucose transport in the soleus muscle, but not in the epitrochlearis, was still significantly elevated (26%) relative to vehicle-treated control.

Conclusions: Oral treatment of insulin-resistant ZDF rats with a novel specific GSK3 inhibitor markedly enhances oral glucose tolerance and whole-body insulin sensitivity, at least in part because of an improvement in insulin action on skeletal muscle glucose transport activity. *In vivo* inhibition of GSK3 activity appears promising as an intervention against the glucose intolerance and insulin resistance of skeletal muscle glucose disposal associated with the ZDF rat.

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Inflammation as a part of the metabolic syndrome

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Background and Aims: Recently chronic subclinical inflammation was suggested to be a part of the metabolic syndrome. The aim of this study was to examine the relationship of inflammatory variables, such as C-reactive protein (CRP), leucocytes count and fibrinogen to parameters of the metabolic syndrome.

Materials and Methods: A total of 782 subjects, aged 40 to 70 years, were analysed from the Risk factors in IGT for Atherosclerosis and Diabetes (RIAD) study. Plasma glucose, lipids, fibrinolytic and coagulation parameters and inflammatory variables were measured by conventional methods; proinsulin and real insulin by highly specific enzyme immunoassays and albuminuria by nephelometry.

Results: Subjects with increased CRP exhibited significantly higher body mass index (BMI), waist to hip ratio (WHR), fasting and postprandial (pp) plasma glucose and glycemic spikes, insulin resistance (HOMA), free fatty acids, insulin (fasting and pp), plasminogen activator inhibitor, fibrinogen and microalbuminuria. Leucocytes count significantly correlated to blood pressure, BMI, WHR, triglycerides, high-density lipoprotein cholesterol, insulin resistance (HOMA), fibrinogen, plasminogen activator inhibitor, tissue plasminogen activator, microalbuminuria and low physical activity, as well as to fasting and pp levels of plasma glucose, proinsulin and specific insulin. Fibrinogen was significantly related to blood pressure, BMI, total cholesterol, triglycerides, insulin resistance (HOMA), plasma glucose, plasminogen activator inhibitor, tissue plasminogen activator and low physical activity.

Conclusions: Our data support the hypothesis that inflammatory processes are interrelated with the metabolic syndrome.

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Metformin corrects insulin resistance induced by chronic hyperinsulinism in HepG2 cells.

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Background: A decreased hepatic glucose output is mainly responsible for the antidiabetic effect of metformin (MET), but the molecular mechanisms involved herein are insufficiently understood. We therefore characterized the effect of MET on insulin signaling in the human hepatoma cell line HepG2, both under regular conditions and in cells in which insulin resistance had been induced.

Methods: HepG2 cells were incubated without or with MET (10 μ M-10 mM) for 16h and/or stimulated with insulin (100 nM) for 1 min. Protein amounts of insulin receptor (IR), IRS1, IRS2 and p85 subunit of PI3-kinase (p85) were determined in total lysates, while phosphorylated IR, IRS1, IRS2 and p85 interaction with IRS1 and IRS2 were detected after immunoprecipitation. For the second set of experiments, cells were incubated with (10 nM) or without insulin for 16h, together with (10 μ M) or without MET. After washing of the cells, acute stimulation with insulin (100 nM) was performed, before amount and phosphorylation of signaling proteins were determined.

Results: Under regular conditions, MET had no effect on total amounts of signaling proteins. 10 and 100 μ M metformin had no significant effect on insulin-stimulated phosphorylation of IR, IRS1, IRS2 and the interaction of IRS1 and IRS2 with p85. Higher concentrations resulted in a significant reduction of activation by 74% (IR), 82% (IRS1) and 73% (IRS2) along with reduced association of p85 with IRS1 (-69%) and IRS2 (-63%; all $p < 0.05$). Chronic preincubation with insulin caused a reduction of insulin's acute effect on phosphorylation of IR (-73%), IRS1 (-78%) and IRS2 (-77%; all $p < 0.05$). Simultaneous preincubation with MET eliminated most of this effect and resulted in a reduction of acute insulin-stimulated phosphorylation of IR by only 20%, of IRS1 by 22% and of IRS2 by 16%. A reduction of the total amount of IR, IRS1 and IRS2 after chronic pretreatment with insulin was almost normalized by metformin. While chronic insulin treatment reduced the p85-association of IRS1 to 30 % and of IRS2 to 34% of control level, coincubation with MET restored the p85-association of IRS1 to 91% and with IRS2 to 92% of control level.

Conclusions: Under normal conditions, increased expression or phosphorylation of insulin signaling proteins does not seem to play a role in the effect of therapeutic MET concentrations on hepatocytes. Pharmacologic concentrations have inhibitory effects on signaling. The suppressing effect of chronic hyperinsulinism on amount and phosphorylation of insulin signaling proteins is almost normalized by metformin in HepG2 cells, indicating how the drug could improve hepatic insulin resistance in type 2 diabetes.

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Basal Glycaemia and Glucose Tolerance is Improved after Resiniferatoxin in male Zucker Diabetic Fatty rats

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Background and Aims: It is known that sensory nerves are of importance for glucose homeostasis and insulin secretion. We have previously demonstrated that sensory denervation accomplished by capsaicin improves glucose tolerance and might prevent deterioration of type 2 diabetes in Zucker Diabetic Fatty (ZDF) rats. Resiniferatoxin, RTX, is an ultra potent capsaicin analogue, efficient after a single and 1000 fold lower dose than capsaicin and is generally better tolerated. No previous studies have investigated RTX' influence on rodent type 2 diabetes and we therefore explored the hypothesis that sensory denervation accomplished by RTX improves type 2 diabetes in male ZDF rats. **Materials and Methods:** Two groups of overtly diabetic male ZDF rats (17 w, n=6) with matched fasting blood glucose (FBG) levels (FBG: 10.9 \pm 1.8 vs. 12.5 \pm 1.7 mmol/L, $p = 0.525$) and oral glucose tolerance (OGTT-AUC-BG: 0-120 min: 2224 \pm 325 vs 2263 \pm 189 min*mmol/L, $p = 0.919$) were given either a single subcutaneous injection of RTX (0.1 mg/kg, sc) or vehicle (VEH: saline and ethanol). Body weights were monitored during two weeks after which an OGTT was performed (samples: 0, 30, 60 and 120 min). Islets of Langerhans were then isolated and used for *in vitro* studies of glucose stimulated insulin secretion.

Results: Data are mean \pm SE (FBG: Student's t-test and AUC: Mann-Whitney U test). Two weeks after RTX or VEH, weight curves were indifferent between groups. However, FBG was decreased after RTX (FBG: 7.5 \pm 0.9 vs 10.0 \pm 0.8 mmol/L, $p = 0.073$) and oral glucose tolerance was improved (OGTT-AUC-BG: 0-120 min: 1366 \pm 218 vs 1873 \pm 121 min*mmol/L, $p = 0.055$). This improvement was paralleled by a higher 30-min insulin response in RTX treated rats (OGTT-AUC-INS: 0.30 min: 23750 \pm 7090 vs 11248 \pm 1585 min*pmol/L, $p = 0.025$). The ratio between blood glucose and plasma insulin for the first 30 min after oral glucose was also significantly lower after RTX (AUC-BG/INS(0-30min): 0.21 \pm 0.006 vs 0.041 \pm 0.006, $p = 0.029$). Glucose stimulated insulin secretion from isolated islets was not affected by RTX. **Conclusions:** Sensory denervation by RTX improves the state of type 2 diabetes in severely diabetic male ZDF rats by reducing basal glycaemia and improving glucose tolerance partly due to a higher insulin secretion. Since a potentiation of the glucose-stimulated insulin secretion is not observed in isolated islets, we suggest that the glycaemic effect observed *in vivo* results after improvement of hormonal and/or neural stimulatory influences on beta cell function following the sensory denervation.

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DECREASED GLUCOSE UPTAKE AND GLYCOGEN SYNTHESIS IN CULTURED HUMAN SKELETAL MUSCLE CELLS AFTER CHRONIC IGF-1-STIMULATION

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The effects of chronic insulin growth factor-1 (IGF-1) stimulation of skeletal muscle cells were studied. Cultured human myoblasts were incubated for a 4 day-fusion period with or without recombinant IGF-1 (60 ng/ml). Acute IGF-1 effects were ended by a serum-free incubation for 5 h. Subsequently, cells were stimulated with or without 100 nmol/l insulin for measurements of insulin signalling, glucose uptake and glycogen synthesis. Chronic IGF-1 treatment increased protein synthesis (1.14 ± 0.03 vs. 0.86 ± 0.03 relative units [relU] per cell culture dish, IGF-1 vs. control, $p < 0.05$) and enhanced myogenic differentiation (creatine kinase activity: 1.20 ± 0.04 vs. 0.80 ± 0.04 relU, $p < 0.05$; a-sarcomeric actin-content: 1.39 ± 0.09 vs. 0.61 ± 0.09 relU, $p < 0.05$, per mg of protein). IGF-1 decreased absolute basal (0.79 ± 0.03 vs. 1.01 ± 0.04 relU, $p < 0.05$) and insulin-stimulated (0.91 ± 0.05 vs. 1.26 ± 0.05 relU, $p < 0.05$) glucose uptake as well as absolute basal (0.53 ± 0.02 vs. 0.77 ± 0.03 relU, $p < 0.05$) and insulin-stimulated (1.08 ± 0.09 vs. 1.62 ± 0.06 relU, $p < 0.05$) glycogen synthesis, but did not influence insulin responsiveness. Chronic IGF-1-stimulation decreased insulin-stimulated serine phosphorylation of protein kinase B (PKB) (1.32 ± 0.08 vs. 2.19 ± 0.08 relU, $p < 0.05$) and PKB protein-content (0.95 ± 0.01 vs. 1.05 ± 0.01 relU, $p < 0.05$). No significant reduction was measured for insulin-stimulated insulin receptor-substrate-1 associated phosphatidylinositol 3'-kinase activity (0.88 ± 0.18 vs. 1.12 ± 0.18 relU, $p = 0.52$). These data suggest that the beneficial effects of IGF-1 treatment in insulin resistant states are not explained by chronic effects of IGF-1 on glucose metabolism in skeletal muscle.

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Chronic Hyperinsulinism Affects Insulin Receptor Intracellular Processing in Cultured Muscle Cells.

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A crucial issue in the interpretation of the derangements of insulin-insulin receptor complex in cells from type 2 diabetic patients is the role played by hyperinsulinemia. We examined the effect of chronic hyperinsulinism (3-6 weeks) on insulin receptor (IR) cellular trafficking in skeletal muscle cells (SKMC), grown in media containing 43-47 pM (N) or 1.07-1.43 nM (H) insulin. Chronic hyperinsulinism decreased significantly insulin-IR binding ($1.8 \pm 0.5\%$ vs $4.3 \pm 0.9\%$, $p < 0.01$) but not hormone internalization. IR expression evaluated by immunoblotting with anti insulin receptor B-subunit antibody was superimposable in N and H. Internalized insulin-IR complex which remains undissociated was higher ($p < 0.05$) after 15 min in SKMC-H ($42.6 \pm 1.16\%$) than in SKMC-N ($30.85 \pm 2\%$) but the values become similar after 45 min. Moreover, SKMC-H showed a complete but slower ($p < 0.05$) recycling of IR back to plasma membrane ($t_{1/2} = 20$ min vs SKMC-N $t_{1/2} = 7$ min). Intracellular insulin degradation was not different in the two experimental conditions. As a consequence the concentrations of total intracellular insulin measured by HPLC were significantly ($p < 0.01$) lower in H than in N cells. We conclude that continuous exposure to high insulin levels induces in cultured SKMC a down regulation of insulin binding and some alterations of intracellular insulin-IR complex processing. These features do not resemble those reported in cells from patients with type 2 diabetes mellitus.

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IN VIVO ¹H-NMR SPECTROSCOPY FOR DETERMINATION OF INTRAMYOCYELLULAR LIPIDS IN ZUCKER DIABETIC FATTY RATS

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Background: There is growing evidence that increased levels of lipids in muscle cells (IMCL) are involved in insulin resistance. The aim of the present study was to use the ¹H-NMR spectroscopy technique at high magnetic field strength (7T) for monitoring IMCL in vivo in ZDF rats over 16 weeks (age: 6 to 21 weeks) and to correlate IMCL to the insulin sensitivity measured by the euglycemic-hyperinsulinemic glucose clamp technique.

Material and Methods: 15 male obese ZDF rats (fa/fa) and 15 lean control rats (+/?) were used. 5 rats per group were clamped at the age of 6 weeks to determine their insulin sensitivity (insulin: 4.8 and 9.6 mU/kg/min). IMCL of the other rats were monitored by ¹H-NMR spectroscopy several times until they were 18-21 weeks old. At the end of the study insulin sensitivity was measured by another glucose clamp study.

Results: At the age of 6 weeks the glucose infusion rate (GIR) in obese ZDF rats was 2.7 and 8.9 mg/kg/min for the low and high insulin infusion rate, respectively (control: 20 and 28 mg/kg/min), indicating insulin resistance in prediabetic obese animals. ¹H-NMR spectroscopy measurement of IMCL at that age demonstrated already a 2.3 times higher IMCL signal in obese animals compared to lean control rats. IMCL increased during the study period up to 10-fold in obese vs lean rats. At the end of the study insulin resistance was confirmed functionally in obese ZDF rats compared to lean control rats.

Conclusion: It is concluded that monitoring of IMCL signals by ¹H-NMR spectroscopy in rats might be a useful method for the longitudinal characterization of new drugs affecting muscle lipid metabolism and thereby influencing insulin sensitivity.

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PREVENTION OF DIABETES IN THE PSAMMOMYS OBESUS BY VANADIUM COMPLEX.

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Aims: To demonstrate the preventive effect of vanadium complex L-glutamic-acid(γ)monohydroxamate (LP-100) on nutritional diabetes in *Psammomys obesus*. **Methods:** Male *Psammomys* aged 3 months, kept on low energy (LE) diet, were placed on high energy (HE) diet. Each animal was returned to LE diet after 3-5 consecutive blood glucose tests were >12 mmol/l. Animals remained on LE diet at least 5 days at which time they returned to normoglycemia (<5 mmol/l). In the second stage the same animals were divided into control and LP-100 groups (n=10) and transferred to HE diet. They received by gavage, water or LP-100, 32 mg/kg, for 7 days with blood glucose levels and weight monitored daily, then kept on HE diet for additional 14 days. The two groups, were compared, each animal serving as its own control at the first stage on HE diet. **Results:** The mean time needed for the *Psammomys* to become hyperglycemic (>12 mmol/l) during the first period on HE diet was 4 days. All animals in the control group developed hyperglycemia during the second period on HE diet, most (7 of 10) at a very similar time. None of animals in the LP-100 group became hyperglycemic during the treatment period. During the post-treatment period animals remained normoglycemic for at least 5 days. There was no change in the mean weight gain during or after the treatment. **Conclusion:** Diabetes in *Psammomys obesus* is induced by HE diet, whereas 7 daily oral doses of 32 mg/kg LP-100 prevented and delayed the development of the diabetic state even when fed the HE diet. The insulin sensitizing vanadium salt protects this animal with innate insulin resistance from nutritionally induced diabetes.

Hyperinsulinemia upregulates phosphodiesterase 3B and inhibits lipolysis in 3T3-L1 Adipocytes: a potential link between insulin resistance and obesity

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Background and Aims: Obesity is often associated with hyperinsulinemia and insulin resistance. The mechanism by which an enlarged adipose tissue mass that defines obesity causes systemic insulin resistance remains uncertain. However, impaired insulin action coupled with hyperinsulinemia has been suggested to lead to a variety of abnormalities, including elevated triglycerides and enhanced secretion of VLDL. Aim of this study is to investigate the potential mechanism of impaired insulin action in adipocytes induced by hyperinsulinemia, with focus on lipolysis and cyclic nucleotide phosphodiesterase 3B, a key enzyme for the antilipolytic action of insulin.

Materials and Methods: Differentiated 3T3-L1 adipocyte was used in this study. The medium was collected for lipolysis assay. The cells were homogenized, the membrane fractions was prepared for determination of the activity and protein expression of PDE3B. **Results:** Incubation of 3T3-L1 adipocytes with 10, 100 and 1000 nM insulin plus high glucose (25 mM) for 24 h resulted in a dose-dependent increase of PDE3B activity and protein expression. Increased PDE3B activity was also found in cells treated with 100 nM insulin plus high glucose for 48 and 72 h (103 ± 3.5 and 137 ± 3 vs 44 ± 2.1 and 67 ± 2.4 pmol/min/mg protein, $p < 0.05$). Treatment of cells with 100 nM insulin plus low glucose (5.5 mM) resulted in an increased activity of PDE3B by 1.28-, 2.1- and 2.2-fold compared to the cells treated with low glucose without insulin. This increased PDE3B activity accompanied an elevation of PDE3B protein expression. However, there was no significant increase in PDE3B activity in the cells cultured either with low glucose or high glucose in the absence of insulin. In agreement with the change of PDE3B, 100 nM insulin plus high glucose significantly decreased the lipolysis after treatment for 24, 48 and 72h (3.5 ± 0.6 vs 1.7 ± 0.11 , 7.2 ± 0.39 vs 3.6 ± 0.4 and 8.9 ± 0.61 vs 4.3 ± 0.32 $\mu\text{mol/mg protein}$, $p < 0.05$) when compared to high-glucose treated cells. Furthermore, treatment of cells with insulin plus low glucose also decreased lipolysis compared to low-glucose treated cells. **Conclusion:** Taken together, these results suggest that the up-regulation of PDE3B by hyperinsulinemia could be one the reasons for increasing adipocyte triglyceride storage, thus promoting insulin resistance in obesity.

Effects of Cholecystokinin octapeptide on the pancreas of type 1 and type 2 diabetic rats.

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Background and Aims: Cholecystokinin (CCK) regulates the release of pancreatic enzymes and modulates the growth of the pancreas. In diabetes, the sensitivity of the pancreas to CCK is decreased. Thus the aim of this study was to investigate the effects of increasing concentrations of CCK-octapeptide (CCK-8) on the pancreatic content and to evaluate the role of the CCK receptor in the loss of sensitivity in diabetic rats.

Materials and Methods: Type 1 diabetes was induced in adult Wistar rats by an I.V. injection of 65 mg/kg streptozotocin. Type 2 diabetes was obtained by an I.P. injection of 260 mg/kg nicotinamide given 15 min before streptozotocin. 14 days thereafter, CCK-8 (1, 2 and 4 mg/kg) or saline (control group) were injected S.C., three times daily, for 8 successive days, in 7 animals per group. After sacrifice, the pancreas was excised for biochemical analysis. CCK receptors were characterized by binding assays.

Results: CCK-8 exerted a biphasic action on the control group as well as on the diabetic group. In the control group, CCK-8 increased significantly the pancreatic weight, its content in proteins, RNA and enzymes (amylase, lipase), with a maximal effect (31, 74, 45, 57 and 46% respectively, $p < 0.001$) observed with 1 mg/kg CCK-8. The dose-inhibition curve of CCK-8 inhibiting binding of ^{125}I -CCK-8 was significantly best fit by a two-site model with high-affinity site ($K_d = 3.03 \pm 0.16$ nM, $B_{\text{max}} = 113.27 \pm 10.3$ fmol/mg protein) and a low-affinity site ($K_d = 90.96 \pm 9.38$ nM, $B_{\text{max}} = 1042.5 \pm 76.47$ fmol/mg protein). In streptozotocin-induced diabetic rats, glycemia was approximately 455 mg/dl. The most marked effect was a strong reduction in pancreatic amylase (-98%, $p < 0.001$) content. CCK-8 administered to these rats also increased pancreatic weight and its content in proteins, nucleic acids and enzymes but the dose-response curve was shifted toward the higher concentrations of CCK-8 (4 mg/kg). In this model, Scatchard analysis was compatible with a one-class of binding site on the pancreas: $K_d = 6.53 \pm 1.180$ nM, $B_{\text{max}} = 2057.43 \pm 68.58$ fmol/mg protein. In nicotinamide treated diabetic rats, glycemia was slightly higher than in normal rats (+26%, $p < 0.05$). The pancreatic amylase content was reduced by 47% ($p < 0.01$). The maximal effect was observed with the concentration of 1 or 2 mg/kg CCK-8, according to the analysed parameter. The data could be best fit to a one-binding site model: $K_d = 103.94 \pm 18.42$ nM, $B_{\text{max}} = 1455.82 \pm 100.33$ fmol/mg protein.

Conclusions: CCK exerts a biphasic growth response on the pancreas of diabetic rats, mediated by one class of CCK-A receptors.

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Glucose and lipid metabolism in obese Black and White South African Type 2 diabetic patients

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Background and Aims: Studies have shown that obese Black women are more insulin resistant and have lower β -cell secretory activity than obese White women. Our objective was to determine whether such differences occur in Type 2 diabetics and to what extent they contribute to differences in disease pathology in Black and White South African Type 2 diabetics.

Materials and Methods: Various metabolic indices and hormone levels were measured at fasting and during a 7-hour OGTT in 8 diabetic Black women (DBW), 7 diabetic White women (DWW), 10 obese non-diabetic Black women (OBW) and 9 obese non-diabetic White women (OWW). In vivo glucose oxidation was evaluated from the level of breath $^{13}\text{CO}_2$ following ingestion of $[1-^{13}\text{C}]$ glucose. Subcutaneous and visceral fat area (VF) were assessed via CT-scans. **Results:** VF was higher in DWW than DBW (184 ± 12 vs 143 ± 14 cm 2 ; $p = 0.05$) and in OWW than OBW (140 ± 11 vs 72 ± 4 cm 2 ; $p < 0.01$) and higher in diabetics than non-diabetics. Insulin resistance (HOMA) showed greater differences between OWW and DWW (2.55 ± 0.58 vs 5.23 ± 0.78 respectively; $p < 0.01$) than between OBW and DBW (3.19 ± 0.39 vs 3.80 ± 0.93 respectively; $p = \text{NS}$). Total (area under the curve [AUC]) insulin levels were lower in DBW than OBW (58 ± 13 vs 115 ± 16 nM respectively; $p < 0.05$) but were not different between DWW and OWW (81 ± 16 vs 113 ± 20 nM respectively; $p = \text{NS}$). Total glucose oxidation was lower in each diabetic group than the respective non-diabetic group ($p < 0.01$ for both) and correlated negatively with visceral fat area ($\beta = -0.49$, $p < 0.01$). Triglyceride levels correlated positively with visceral fat area ($\beta = 0.53$, $p < 0.01$). Total lactate levels were higher in DWW (680 ± 140 mM; $p < 0.01$ vs OWW and $p < 0.05$ vs DBW) and DBW (470 ± 30 mM; $p < 0.05$ vs OBW) than OWW (290 ± 10 mM) and OBW (310 ± 20 mM). **Conclusions:** The progression from obesity to type 2 diabetes in the Black population seems to involve a greater fall in β -cell function than in White subjects in whom an increase in insulin resistance seems more important. In both populations Type 2 diabetes leads to a fall in glucose oxidation which is associated with an increase in visceral fat area. Non-oxidative glucose metabolism as assessed from plasma lactate levels was higher in the diabetics. This is probably a result of the mass effect of high blood glucose levels in combination with increased insulin resistance at the level of pyruvate dehydrogenase. Visceral fat has negative influences on β -cell activity and intermediary metabolism in both populations.

EFFECTS OF WEIGHT REDUCTION ON INSULIN RESISTANCE AND GLUCOSE TOLERANCE WITH ORLISTAT TREATMENT IN OBESE PATIENTS

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Background and aims: Orlistat (ORL) is a gastrointestinal lipase inhibitor that reduces dietary fat absorption, promotes weight loss and may reduce the risk of developing type 2 diabetes mellitus. Our aim was to compare the efficacy of ORL in conjunction with hypocaloric diet versus hypocaloric diet alone in weight loss, in insulin resistance (IR) and glucose tolerance status. **Materials and methods:** A total of 256 obese patients (mean age 48.2 ± 2.1 years) with a body mass index (BMI) of $30-46$ kg/m 2 were evaluated in randomized, clinical trial of two groups; ORL 120 mg tid plus hypocaloric diet (n:154) and hypocaloric diet alone (500-800 kcal daily deficit) (n:102). All the patients were followed-up to twelve months. A standard 2-hour oral glucose tolerance test (OGTT) was performed at the beginning and at the end of treatment. Fasting glucose and insulin values were monitored to measure IR index using the homeostasis model assessment (HOMA-IR) formula. Changes in body weight, glucose tolerance status and IR index were measured. **Results:** Subjects who were treated with ORL plus hypocaloric diet lost more weight (mean \pm SD, 7.23 ± 0.58 kg from initial weight) than subjects who received hypocaloric diet alone (3.24 ± 0.41 kg; $p < 0.001$). A smaller percentage of subjects with impaired glucose tolerance (IGT) at baseline progressed to diabetes status in the ORL (2.5%) vs hypocaloric diet alone (6.2%) group. Conversely, among subjects with IGT at baseline, glucose levels normalized in more subjects after ORL treatment (46.5%) vs hypocaloric diet alone (32.2%; $p < 0.05$). HOMA-IR was improved more with ORL treatment (from 3.12 ± 0.26 to 2.56 ± 0.23 (17%) vs from 3.03 ± 0.27 to 2.89 ± 0.21 (4.6%); $p < 0.001$). **Conclusions:** Orlistat in conjunction with a hypocaloric diet promotes a significant weight loss in obese patients and contributes to improve the glucose tolerance status and IR than diet alone.

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Variants in the genes encoding UCP1 and UCP3: potential role in early-onset obesity.

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Background and Aims: Uncoupling proteins (UCPs) constitute a family of mitochondrial transmembrane carriers involved in the regulation of energy homeostasis. Once activated, they facilitate the dissipation of the electrochemical proton gradient across the inner mitochondrial membrane, thereby allowing stored energy to be converted to heat. While UCP1 is uniquely expressed in brown adipose tissue, UCP is mainly found in skeletal muscle. Genetically determined differences in the expression of UCP1 and/or UCP3 could affect thermogenesis and predispose an individual towards the development of obesity. We therefore investigated the possible association of polymorphisms in the regulatory sequence of the UCP1 and UCP3 genes with early-onset obesity. **Materials and Methods:** Genomic DNA was obtained from a cohort of 294 extremely obese children and adolescents and from a group of 134 underweight control subjects. Genotyping was performed by PCR amplification with specific oligonucleotides, restriction enzyme digestion and agarose gel electrophoresis to assess allele and carrier frequency of the A/G variant at position -3826 in the promoter of the UCP1 gene and the C/T variant at position -55 bp in the promoter of the UCP3 gene. **Results:** The G allele in the UCP1 promoter was found with a frequency of 0.273 in obese and 0.184 in lean subjects ($p = 0.017$). Carriers of the G allele (homozygotes + heterozygotes) were significantly more frequent among obese children and adolescents compared to lean controls (49.2 % vs. 36.9 %, $p = 0.018$). In the promoter of the UCP3 gene, the T allele was found with a frequency of 0.226 in obese and 0.309 in lean subjects ($p = 0.012$). Homozygous + heterozygous carriers of the T allele in the UCP3 gene were significantly more frequent among lean compared to obese individuals (54.5 % vs. 38.8 %, $p = 0.0033$). **Conclusions:** The variant G allele at -3826 bp in the UCP1 gene was found to be associated with early-onset obesity. In contrast, the variant T allele at -55 bp in the UCP3 promoter was found at a markedly lower frequency in obese compared to lean subjects, suggesting a role of the 'wildtype' C allele for obesity predisposition in childhood and adolescence. Our data suggest that polymorphisms in the UCP1 and UCP3 genes are involved in energy homeostasis and body weight regulation, potentially via an impact on UCP1 and UCP3 gene expression.

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IDENTIFICATION OF NOVEL STOMACH GENES INVOLVED IN THE DEVELOPMENT OF OBESITY AND DIABETES

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Background and Aims: Several peptides expressed in the stomach in response to feeding or distention are known to be involved in the regulation of food intake. We hypothesise that there are multiple signals acting between the gastro-hypothalamic axis to regulate feeding behaviour. The aim of this study was to identify differentially expressed genes within the stomach between groups of animals that were subject to varying degrees of distention induced by ad libitum feeding, fasting and refeeding after a fast. To do this we utilised *Psamomys obesus*, a unique animal model of obesity and type 2 diabetes. **Materials and Methods:** Animals were divided into 3 groups: Group 1 ($n=15$) were fed ad libitum, Group 2 ($n=14$) were fasted for 16 hours, Group 3 ($n=13$) were fasted for 16 hours followed by 1 hour ad libitum access to food. This achieved varying levels of stomach distention as evidenced by stomach content weight (Group 1 2.57 ± 0.06 g, Group 2 1.48 ± 0.07 g, Group 3 3.76 ± 0.14 g, $p < 0.001$ by ANOVA). RNA was extracted from the upper area of the stomach and utilised for differential display RT-PCR. **Results:** To date, 5 genes have been confirmed as being differentially expressed using Sybr Green™ real-time PCR and are undergoing further investigation. Of these genes, 3 demonstrated increased expression and 2 showed decreased expression with stomach distention. **Conclusions:** The identification and characterisation of these genes may clarify their role in the regulation of food intake, and provide a potential therapeutic target for the treatment of obesity.

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Genes on chromosome 4 determine an incomplete metabolic syndrome: Lessons from BB.LL rats.

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Background and Aims: The lymphopenia (ll) is essential for development of insulin-dependent diabetes mellitus in the BB rat. To study the phenotypic consequence of a non-lymphopenic BB rat, the region containing the lymphopenia gene of the BB/OK rat was replaced by that of the non-lymphopenic and hypertensive rat, SHR, (11cM, D4Mit6-LL-Npy-Spr). The resulting congenic strain termed BB.LL was non-lymphopenic, did not develop diabetes and showed significantly elevated body weight and serum lipids at an age of 12 weeks compared with BB/OK. That prompted us to study longitudinally BB.LL and BB/OK.

Materials and Methods: 12 BB.LL and 12 BB/OK male rats were studied for traits with pathophysiological relevance to the metabolic syndrome from 3rd to 15th months. **Results:** Most traits studied were significantly different between both, BB.LL and BB/OK and showed age dependence. At an age of 15 months BB.LL rats were markedly heavier than BB/OK (590 ± 28 vs. 485 ± 30 g). Serum leptin (14.8 ± 2.7 vs. 4.6 ± 1.2 ng/ml), triglycerides (2.8 ± 0.4 vs. 1.4 ± 0.2 mmol/l), total cholesterol (5.0 ± 0.4 vs. 4.2 ± 0.7 mmol/l) and insulin (3.5 ± 1.2 vs. 1.4 ± 0.5 ng/ml) were significantly higher in BB.LL than BB/OK. Despite higher values at an age of 12 weeks, no significant differences were found between BB.LL and BB/OK in urea excretion of total protein (22 ± 11 vs. 37 ± 27 mg/24h) and in the creatinine clearance (0.41 ± 0.06 vs. 0.44 ± 0.08 ml/min/100g) at an age of 15 months.

Conclusions: These findings clearly show 1) that congenic BB.LL rats develop an incomplete metabolic-like syndrome and 2) that within the transferred region one or more gene(s) must be located causing this phenotype.

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RELATIONSHIP BETWEEN URIC ACID AND VISCERAL FAT IN OBESE AND NON-OBESE TYPE 2 DIABETIC PATIENTS

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Background and Aims: Hyperuricemia is suggested as an additional component of the metabolic syndrome. The aim of this study was to investigate possible associations between uric acid metabolism and body composition in obese and non-obese type 2 diabetic patients. **Materials and Methods:** Parameters of uric acid metabolism, glucose metabolism and body composition were assessed in 152 type 2 diabetic patients (65 men and 87 women). **Results:** Mean serum uric was 5.1 ± 1.3 mg/dl, mean 24-hour urinary acid excretion was 627 ± 246 mg/dl. All 24 patients with serum uric acid levels above 7.5 mg/dl were overweight ($BMI > 25$ kg/m²). Of the 39 patients with urinary uric acid excretion higher than 750 mg/dl, all but two were overweight. These patients with an elevated urinary excretion had significantly higher total fat mass, fat free mass, and more visceral fat than the patients with normal uric acid excretion, while no difference in subcutaneous fat was seen. Serum uric acid was correlated with weight ($r=0.22$), BMI ($r=0.27$) and fat mass ($r=0.21$) (all $p < 0.01$), but not with fat free mass ($r=0.01$). Strong correlations were found between urinary uric acid excretion and weight ($r=0.46$), BMI ($r=0.32$), fat free mass ($r=0.39$) (all $p < 0.001$) as well as fat free mass ($r=0.23$; $p < 0.05$). Significant correlations were found between serum uric acid and fasting insulin ($r=0.27$), but no correlation between uric acid excretion and insulin levels was found. **Conclusions:** Hyperuricemia occurs in 15.7%, and increased urinary acid excretion in 24.3% of overweight type 2 diabetic patients. Both seem to depend on body composition: serum uric acid being associated with BMI, urinary excretion with fat mass as well as fat free mass.

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INTRABDOMINAL FAT MEASURED BY ULTRASOUND ESTIMATES THE METABOLIC SYNDROME BETTER THAN WAIST CIRCUMFERENCE.
R.P. Stolk, W.P.Th.M. Mali, Y. van der Graaf, on behalf of the SMART study group (Secondary Manifestations of ARterial disease). Julius Center for Patient Oriented Research, University Medical Center Utrecht, the Netherlands.

Background

Recently we developed an ultrasound technique to assess the amount of intra-abdominal fat which is more accurate than waist/hip circumferences, and simpler than CT/MRI scanning.

Methods

This ultrasound measurement was performed in 171 consecutive participants of the SMART study, a cohort study in all patients who presented for the first time with a cardiovascular disorder or cardiovascular risk factor at the UMC Utrecht. Mean age was 56.1 years, 32.2% were women, mean BMI was 27.1 kg/m².

Results

Intra-abdominal fat increases with age (ultrasound: $r=0.35$, $p<0.001$, waist/WHR: $r=0.21$, $p=0.01$). Women have less intra-abdominal fat (both ultrasound and waist/WHR $p<0.001$). There was no association between BMI and age or gender.

Increased intra-abdominal fat was associated with increased plasma triglyceride, total cholesterol, HDL-cholesterol and glucose levels. The partial correlation coefficients, adjusted for age and gender, of the ultrasound measurement were 0.45 ($p<0.001$), 0.19 ($p=0.01$), -0.32 ($p<0.001$), and 0.29 ($p<0.001$), respectively. The coefficients of waist/WHR were 0.24 ($p=0.01$), 0.10 ($p=0.28$), -0.25 ($p=0.01$), and 0.39 ($p<0.001$), respectively.

Conclusion

These results confirm the findings of CT/MRI investigations that intra-abdominal fat increases with age and is strongly associated with metabolic risk factors. The associations are stronger when ultrasound measurements are used, which suggests that the amount of intra-abdominal fat can be more reliably assessed by ultrasound than by waist circumference or WHR.

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SHORT- AND LONG-TERM RESPONSES TO VLCD IN OBESE PATIENTS WITH TYPE 2 DIABETES IN SECONDARY FAILURE

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Background & Aims: The best management for obese patients with type 2 diabetes (T2DM) in secondary failure is unclear, but very-low-calorie diets (VLCDs) are often not considered because of concerns about safety/supervision and weight regain. To evaluate the effects of VLCDs on weight, glycaemic control and cardiovascular risk factors in obese patients with poorly controlled type 2 diabetes.

Methods: Forty patients with symptomatic T2DM (mean age 52 yrs, wt 115kg, BMI 40, Fructosamine 389) in secondary failure undertook 2-months of VLCD therapy (SlimFast VLCD + fruit & vegetables, 700kcal/day). Cardiovascular risk factors, symptom status and metabolic control were assessed at baseline and after 2-months ($n=40$), and follow-up data at 1 year are available for the first 20 patients.

Results: VLCD treatment was well tolerated with no patient withdrawals. Diabetic symptoms, and both oral and insulin therapy requirements, decreased substantially. Average wt loss was 12kg after 8 wks, and mean BMI fell from 40.0 ± 5 to $36.0 \pm 4.9 \text{ kg/m}^2$. Additional changes after 2 months: BP $152/82$ to $139/76 \text{ mmHg}$; Fructosamine 389 to 341 ; and total cholesterol 6.0 to 5.4 mM . After 1 year ($n=20$), there were still worthwhile benefits: mean wt and BMI were $107.3 \pm 15.0 \text{ kg}$ and $36.8 \pm 4.4 \text{ kg/m}^2$, respectively, and none had started insulin.

Conclusion: VLCD therapy is safe and effective for dietician use in obese T2DM patients in secondary failure. Short-term effects on symptoms and CHD risk are impressive, and residual benefits are still apparent 1-yr later. Annual pulsed VLCD therapy may be an option for some patients.

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TUNGSTATE TREATMENT REDUCES BODY WEIGHT IN DIET-INDUCED OBESITY

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Background and Aims: Oral administration of sodium tungstate (Na_2WO_4) to experimental animal models of diabetes normalises glycaemia. The amelioration of the diabetic phenotype is accompanied by a significant decrease in body weight (bw) without modifying energy intake (ei). **Aims:** a) to investigate the effectiveness of Na_2WO_4 as an anti-obesity agent in an intact model of rat dietary obesity and b) to determine the putative involvement of uncoupling proteins (Ucp) on this effect.

Material and Methods: Obesity was induced in Wistar rats by feeding with a 'cafeteria diet' for 30 days and maintained with this diet until the experiment was completed. Afterwards animals were treated with 2 g $\text{Na}_2\text{WO}_4/\text{l}$ (drinking fluid) ($n=12$) or untreated ($n=13$) for 32 days. Following treatment, rats from each experimental group were submitted to a recovery period for 35 days. Ei and bw of all animals were recorded daily. Faeces lipid content, blood glucose, insulin, triglycerides (TG), free fatty acids (FFA) and leptin plasma levels were determined at the end of the treatment. Ucp1 and Ucp3 gene expression was analysed in interscapular brown adipose tissue (iBAT) and gastrocnemius muscle (gM) by Northern Blot. **Results:** Na_2WO_4 treatment decreases body weight gain significantly (289.4 ± 29.0 vs $409.8 \pm 16.2 \text{ g}$, $p<0.05$) without modifying neither ei (9610 ± 265 vs $9930 \pm 341 \text{ Kcal/Kg bw}$) nor faeces lipid content (12.8 ± 0.7 vs $12.1 \pm 0.7\%$). Moreover, TG (211.0 ± 46.3 vs $379.6 \pm 40.8 \text{ mg/dl}$), FFA (0.6 ± 0.1 vs $0.9 \pm 0.1 \text{ nmol/l}$) and insulin (2.5 ± 0.9 vs $3.5 \pm 0.8 \text{ ug/l}$) plasma levels were significantly lower in treated rats ($p<0.05$). No differences were observed in blood glucose (107.9 ± 3.3 vs $108.9 \pm 2.4 \text{ mg/dl}$) and leptin (1.7 ± 0.1 vs $1.3 \pm 0.2 \text{ ng/ml/g}$ white adipose tissue) plasma levels between treated and untreated groups. On the other hand, Na_2WO_4 treatment increases mRNA levels of Ucp3 in gM (63%) and iBAT (16%), likewise Ucp1 in iBAT (35%). Recovery period rats increase its body weight fastly, suggesting a reversibility and no toxic effects of treatment. **Conclusions:** These results suggest that Na_2WO_4 could be a useful agent to treat diet-induced obesity. Gene expression changes of Ucp1 and/or Ucp3 may play a role in the bw reduction induced by Na_2WO_4 treatment.

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ADIPOSE TISSUE BLOOD FLOW AND GLUCOSE UPTAKE ARE DECREASED IN OBESITY

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Background and aims: Reduced glucose uptake has shown to exist in isolated adipocytes from insulin-resistant animals and humans. In the present study we determined how insulin resistance in obesity influences perfusion and metabolism in subcutaneous and visceral adipose tissue in vivo.

Materials and methods: Blood flow and glucose uptake (GU) were measured with [^{15}O]-H₂O, [^{18}F]-FDG and PET in 10 obese male subjects (age 32 ± 2 years and BMI $30 \pm 1 \text{ kg/m}^2$) and 10 healthy age-matched (BMI $23 \pm 1 \text{ kg/m}^2$) subjects during euglycemic hyperinsulinemic conditions (serum insulin $\sim 70 \text{ mU/L}$). Magnetic resonance images were used for adipose tissue localisation.

Results: As compared to non-obese subjects, whole body GU was decreased by 36 % ($p<0.01$), subcutaneous GU by 70% (5.2 ± 0.4 vs. $17.6 \pm 3.4 \text{ mmol/kg tissue min}$, $p<0.01$) and visceral GU by 59% (13.2 ± 1.3 vs. $32.4 \pm 3.4 \text{ mmol/kg tissue min}$; obese vs. non-obese, $p<0.01$). There was a correlation between whole body glucose uptake and GU in subcutaneous ($r = 0.68$, $p < 0.05$) and visceral fat ($r = 0.78$, $p < 0.05$). Simultaneously, subcutaneous adipose blood flow was 38% (2.8 ± 0.2 vs. $4.5 \pm 0.7 \text{ ml/100g min}$; $p < 0.05$) and visceral adipose flow 47% lower in obese than in non-obese (3.1 ± 0.3 vs. $5.9 \pm 1.1 \text{ ml/100g min}$; $p < 0.05$). Adipose tissue flow correlated with adipose GU both in subcutaneous ($r = 0.68$, $p < 0.05$) and visceral deposits ($r = 0.58$, $p < 0.05$).

Conclusions: This data shows that insulin resistant obese men exhibit insulin resistance in adipose tissue, which appears as decreased blood flow and GU in subcutaneous and visceral abdominal tissue.

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Correlation between insulin resistance and left ventricular mass in uncomplicated obesity

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Background and Aims: Obesity is a heterogeneous metabolic syndrome in which insulin resistance is a key feature but not always present. In the uncomplicated obese subjects with insulin resistance cardiac morphology and function may be different than in those with preserved insulin sensitivity. In the present study we evaluated the influence of insulin on Left Ventricular Mass (LVM) and LV geometry in uncomplicated obesity. **Materials and Methods:** We selected 40 obese patients (BMI >30 Kg/mq), 29 women, mean age 38±10, BMI 39±6.5 Kg/mq, with normal blood pressure (BP), glucose tolerance, plasma lipids and with a history of fat excess of at least 10 years. 21 lean normal subjects (12 women, mean age 32.7±10.4, BMI 23.1 ±1.4 g/mq) formed the control group. Each subject underwent euglycemic insulin clamp (7 pmol min Kg) associated with indirect calorimetry to evaluate insulin sensitivity (M index) and Resting Metabolic Rate (RMR), bioelectrical impedance analysis to estimate Free Fat Mass (FFM), waist hip ratio (WHR) measurement and echocardiogram to calculate LVM, LVM indexed for FFMkg (LVM/FFMkg) and body surface area (LVMi), LV diastolic and systolic diameters (LVEDD, LVESD). **Results:** The M index value of 21 normal subjects was 7.34±0.32 mg Kg min. We used M index of 6.34 mg Kg min (7.43 -3sd) as threshold of insulin resistance for obese patients. According to these criteria we obtained two groups of obese subjects. 29 obese patients were insulin resistant (IR) with M index= 4.4±1.31 mg Kg min, 11 obese subjects were classified with normal insulin sensitivity (IS) with M index =8.03 ±1.52 mg Kg min. IR obese patients had higher LVM, LVMi, LVM/FFMkg (p =0.001, p =0.001, p =0.05 respectively) than IS patients; LVEDD and LVESD were also greater in IR compared to IS subjects (p = 0.005, p =0.03 respectively). M index and LVM were inversely correlated (R = - 0.520 p =0.006). In a multivariate analysis M index and FFM were the only independent correlates to LVM. IR showed slightly higher BMI than IS subjects (37.2±10.7 vs 34.1±4.0 p =0.03). No differences in age, WHR, BP and RMR between IR and IS obese groups were observed. **Conclusions:** Uncomplicated obesity is characterized by a great variability of the insulin sensitivity degree. Our data show that insulin resistance induces an increase of LVM, remaining into normal range, and precocious changes of LV geometry. True LV hypertrophy probably occurs in obesity only if insulin resistance is associated with hypertension

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IS BEACON, A NOVEL PEPTIDE WITH A ROLE IN OBESITY – AN INTRACELLULAR OR A SECRETED PROTEIN?

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Background and Aims: Beacon, discovered in *Psammomys obesus*, a unique polygenic animal model for diet induced obesity and diabetes, is a novel neuropeptide found to be involved in the regulation of energy balance. In this animal model, positive correlation was observed between the level of beacon gene expression in the hypothalamus and the percent body fat. Beacon is a small protein (73aa) and is highly conserved between species. Peptide hormones such as insulin or NPY or peptides of melanocortin system are synthesised as large precursors and carry a signal sequence for secretion. The sequence of events involving their synthesis, storage, conversion from inactive precursor form to active peptides and release from intracellular stores occurs in distinct stages and each step of the process is highly regulated. In contrast, beacon has no predictable signal sequence for secretion, nor transmembrane regions nor glycosylation sites. To better understand beacon's role in the regulation of energy balance, we have designed experiments to address the question whether beacon is a cytoplasmic intracellular protein as predicted by bioinformatics or an actively secreted protein. **Materials and Methods:** The beacon cDNA has been cloned into mammalian expression vectors with and without epitope tags. Beacon expression in different cells was analysed with antibodies to the tags or beacon itself. **Results:** Mammalian cells expressing c-myc and HA tagged beacon stained intensely with antibodies to the tags. Cell lysates on Western blots revealed a distinct immunoreactive band of expected size (~9 kDa) with anti-beacon and anti-tag antibodies. In addition both types of antibodies consistently detected protein bands of ~15, 22, 40, 50 kDa molecular weights. In the culture medium, beacon protein was undetectable compared to a positive control. **Conclusions:** Beacon expressed in the cells is predominantly intracellular, results indicate its association with other proteins. It is possible that beacon's actions are mediated from within the cell. On the other hand, as with other hormones mobilisation of beacon into extracellular space could be a highly regulated process and requires special physiological conditions. Future studies will examine this aspect in more detail.

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Influence of Ethnicity and Familial Diabetes on Peripheral and Hepatic Insulin Action

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Background and Aims: Risk of developing type 2 diabetes is higher in some ethnic groups, and familial diabetes (FHD) generally enhances the risk. Our aim was to test the hypothesis that insulin resistance - peripheral or hepatic - is the clinical phenotype expressing such risks. **Materials and Methods:** We studied 268 Mexican-American [MA] and 95 Caucasian [C] subjects living in San Antonio, TX. All subjects underwent a 75-g OGTT, and were classified as normal glucose tolerant (NGT, 96 MA and 47 C), impaired glucose tolerant (IGT, 42 MA and 7 C) or diabetic (D, 146 MA and 41 C). On a separate day, all subjects received a [3H]-glucose infusion for the measurement of fasting endogenous glucose output (EGO). In a subgroup of 269 subjects of either ethnicity, insulin sensitivity (M) was measured by a hyperinsulinaemic euglycaemic clamp (40 mU.min⁻¹.m⁻²) on another day. **Results:** While fasting plasma glucose was comparable in MA and C (5.1±0.1 vs 5.1±0.1 mM NGT; 5.3±0.1 vs 5.3±0.1 IGT; 9.0±0.2 vs 9.4±0.4 D), fasting plasma insulin was consistently higher in MA than C both in the fasting state and during the OGTT. EGO was similar in MA and C across glucose tolerance status (15.5±0.2 vs 15.5±0.4 vs 15.8±0.2 μmol.min⁻¹.kgFFM⁻¹). When estimated as the product of EGO and fasting insulin, hepatic insulin resistance was highest in D and intermediate in IGT as compared to NGT (p<0.01) after adjustment for age and BMI (47% of variability explained), but neither ethnicity nor FHD were significant correlates. In contrast, age- and BMI-adjusted M was significantly lower in MA than C (p<0.01) across glucose tolerance status (lowest in D, intermediate in IGT), while FDH was independently associated with lower M in C but not MA (53% of variability explained). **Conclusions:** In Mexican-Americans, peripheral insulin resistance with compensatory hyperinsulinaemia is more severe than in Caucasians living in the same area even in the absence of FHD. Hepatic insulin resistance is worsened by obesity and diabetes, but is not affected by ethnicity or FHD. Peripheral insulin resistance is the phenotypic expression of the higher risk for type 2 diabetes of Mexican-Americans of Southern Texas.

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RATES OF INSULIN SECRETION AND PERIPHERAL GLUCOSE DISPOSAL AFTER A MIXED MEAL IN PATIENTS WITH TYPE 2 DIABETES

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Background and Aims: In type 2 diabetes (NIDDM), there are defects in secretion and peripheral action of insulin. To evaluate their relative contribution to the pathogenesis of hyperglycemia, we assessed postprandial (a) insulin secretion, and (b) insulin effects on glucose disposal (GFLUX) in muscle (M) and adipose tissue (AD). **Patients and Methods:** A standard mixed meal was given to 14 NIDDM patients (age 51±3yrs, BMI 25±0.8, fasting blood glucose 7.8±0.3mM, HbA_{1c} 7.3±0.1%) rendered euglycemic overnight with i.v. insulin, and 6 controls (C, age 47±5yrs, BMI 27±1). Plasma samples for measurements of glucose (G) and insulin (I) were taken for 360min from veins (V) draining the anterior abdominal subcutaneous AD depot and the forearm M, and from an arterialized hand vein (A). With each blood sample, blood flow (BF) was measured in AD (with ¹³³Xe) and M (with strain-gauge plethysmography). Calculations: GFLUX=[G(A-V)] x [BF]; Clearance of G (GCL)=[GFLUX/G in A]; G fractional extraction (GF)=[G(A-V)/G in A]. Postprandial changes are presented as area under curve (0-360 min). **Results:** In NIDDM vs C: (1) Preprandial arterialized G (5.8±0.3 vs 5±0.3 mM) and I (45±6 vs 40±13 pM) were not different. (2) Postprandial increases of arterialized G were higher (3.7±0.27 vs 1.97±0.05 μMmin, p<0.05); those of I – because of disappearance of 1st phase insulin secretion and increase of 2nd – were not different (74±10 vs 72±12 nMmin). (3) Postprandial BF (1766±133 vs 1978±165 ml/100g.tissue in M, and 1084±146 vs 1138±157 ml/100g.tissue in AD), and GFLUX (1026±109 vs 872±40 μmol/100g.tissue in M and 538±97 vs 442±27 μmol/100g.tissue in AD) were not different. (4) Postprandial GCL and GF in M were lower (25±9 vs 139±30 ml/100g.tissue, p<0.05, and 22±3 vs 41±5 %, p<0.05, respectively); respective values in AD were not different. **Conclusions:** In NIDDM, in the postprandial state, there are defects both in the secretion and action of insulin in muscle. The latter can be corrected, at least in part, by hyperglycemia.

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PLASMA TISSUE PLASMINOGEN ACTIVATOR IS HIGHER IN SOUTH ASIANS VERSUS EUROPEANS AND ASSOCIATED WITH THE INSULIN RESISTANCE SYNDROME IN BOTH ETHNIC GROUPS

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Background and Aims: Elevated plasma concentration of the haemostatic factor, endogenous tissue plasminogen activator (t-pa), is predictive of future coronary heart disease (CHD) in European descent populations. South Asians (SA) are a group of people with as yet unexplained high risk of insulin resistance, diabetes and CHD. It is not known if SAs have elevated t-pa and what its correlates are. We wanted to test if t-pa is (i) elevated in healthy SAs relative to Europeans, and (ii) associated with body fat distribution and insulin resistance.

Materials and Methods: We performed a cross sectional study in London. In 111 healthy SA and European men and women aged 40-55 years over a wide range of BMI (17 - 34 kg/m²) we measured percent body fat (DEXA scan), visceral and subcutaneous fat (single slice abdominal CT scan), anthropometry, glucose, insulin, lipids (fasting and postprandial), and t-pa.

Results: T-pa was elevated in SAs [mean (sem) 10.6 (0.6) ng/ml] vs. Europeans [8.2 (0.4) ng/ml], $p=0.001$. This ethnic difference was significant in both men and women ($p=0.026$ and $p=0.011$ respectively). T-pa was significantly positively correlated ($p<0.05$ - <0.0001) in both ethnic groups with fasting insulin, fasting glucose, fasting and 8h triglyceride, total cholesterol, systolic and diastolic blood pressure, BMI, waist girth and waist/hip ratio, visceral fat but not subcutaneous abdominal fat or total percent fat. In multivariate analyses adjusted for age, sex and smoking the ethnic difference in t-pa [$\beta=2.33$, $se=0.82$, $p=0.005$, adjusted $R^2=16\%$] persisted after further adjustment for all the metabolic and fat distribution variables [$\beta=2.12$, $se=0.73$, $p=0.005$, adjusted $R^2=38\%$].

Conclusions: These novel results suggest that (i) South Asian ethnicity is independently associated with elevated t-pa concentration in both men and women, and (ii) t-pa is a correlate of the insulin resistance syndrome in both ethnic groups. Future studies should examine the role of the haemostatic factor t-pa in the increased risk for insulin resistance, diabetes and CHD in South Asians.

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Insulin resistance, beta-cell dysfunction and non-obesity are characteristic in Japanese Type 2 diabetes

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Background and Aim: There is a growing number of diabetic patients in Japan, which is estimated to be over 7 in 1999. Many of the Type 2 diabetes are non-obese, implying the possible pathogenetic contribution of beta-cell dysfunction. The study was designed to evaluate the roles of insulin resistance and beta-cell function in Japanese Type 2 diabetic patients.

Patients and Methods: One-hundred and four Japanese Type 2 diabetic patients with normal kidney function (43 women and 61 men, Age 54.7 [51.8 - 57.6] years old, known diabetes duration 7.4 [6.1 - 8.7] years, body mass index [BMI] 23.5 [22.7 - 24.3] kg/m², mean [95 % confidence interval]) and 10 healthy control subjects participated in the study. We assessed the insulin resistance by determining the steady state plasma glucose (SSPG) and beta-cell function by measuring serum C-peptide level after intravenous dose of glucagon.

Results: The mean SSPG was 12.3 (11.5 - 13.1) mmol/l, and 87.5 % of patients exceeded 6.8 mmol/l, the level 2 SD higher than the mean of 10 healthy subjects. The 6-minute serum C-peptide after glucagon was 1.19 (1.06 - 1.33) nmol/l, and the levels in 90.1 % of patients were below 1.95 nmol/l (the reported mean level in non-diabetic Japanese subjects). About 14.5 % of the patients showed no response to glucagon (<0.60 nmol/l). SSPG correlated with serum free fatty acids significantly ($R_s = 0.309$, $p = 0.0022$), but not with BMI. Urinary C-peptide excretion was the most predictive indicator for insulin treatment.

Conclusions: The results suggest that Japanese Type 2 diabetic patients, living in Japan, are characterized by increased insulin resistance, relative beta-cell dysfunction and non-obesity. Serum free fatty acids level may be causally related to the insulin resistance in these patients.

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BETA-ADRENERGIC RECEPTOR AGONISTS INHIBIT GENE EXPRESSION AND SECRETION OF THE ADIPOCYTE-SPECIFIC SECRETORY PROTEIN, ACRP30

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Background and Aims: ACRP30, the adipocyte complement-related protein (30kDa) is a secreted protein expressed exclusively in differentiated adipocytes. This novel hormone controls body weight, lipid and glucose metabolism in mice. Although catecholamines play a major role in fuel homeostasis, the regulation of ACRP30 by these hormones has not yet been explored. In this study, we investigated the regulation of ACRP30 gene expression, content and secretion in mouse in vivo and in vitro. **Materials and Methods:** Explants from visceral and subcutaneous adipose tissue were cultured in MEM supplemented with 0.5% BSA and the indicated agents for up to 10 h. For in vivo studies, mice were injected with BRL37344 dissolved in saline (2 mg/kg body weight s.c., twice at 8 h interval) and control mice received the vehicle only. mRNA levels of ACRP30 were measured by Northern-blot and protein concentrations by Western-blot analysis. **Results:** We first tested cAMP, the second messenger of catecholamines. The addition of 1mM cAMP to the culture medium for 10 h strongly (by 90%) decreased ACRP30 mRNA levels in explants from both visceral and subcutaneous adipose tissue. This was accompanied by a similar decrease of ACRP30 content in tissue homogenates (mainly at the expense of the cytosol fraction) and by a 50 % reduction of ACRP30 secretion. These effects were time-dependent, occurring from 3 h (mRNA) and 6 h (protein) of culture onwards. The addition of actinomycin D, an inhibitor of transcription, to the medium did actually increase ACRP30 mRNA. This suggests that cAMP exerts its inhibitory effect mainly by decreasing the stability of ACRP30 mRNA. The in vitro effect of cAMP was partly reproduced by isoproterenol, a non-specific beta-adrenergic agonist or BRL 37344, a selective beta3-agonist (60% and 45% decreases of ACRP30 mRNA after 10 h with 10 μ M isoproterenol or 10 μ M BRL, respectively). Administration of BRL 37344 to mice caused a 40-50 % reduction of ACRP30 mRNA levels in visceral and subcutaneous adipose depots and a 40 % decrease in plasma ACRP30 levels. **Conclusions:** The decrease in ACRP30 may be a novel, beta-receptor-mediated mechanism, by which catecholamines affect fuel homeostasis.

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SYMPATHETIC NERVOUS SYSTEM ACTIVITY IN OBESE SUBJECTS WITH INSULIN RESISTANCE AND HYPERINSULINAEMIA

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Knowledge on the sympathetic alterations in the obese state is still controversial.

Hyperkinetic circulation is a reliable marker of increased sympathetic tone. **Aim of study:** to evaluate heart rate and norepinephrine (NA), epinephrine (A) and dopamine (DA) plasma levels in obese subjects characterized by different degree of insulin resistance and hyperinsulinaemia. **Material and methods:** investigation was conducted in 3 groups of obese, borderline hypertensive subjects: 11 newly diagnosed diabetics (DM), 14 insulin resistant (IR) non-diabetic subjects and 14 insulin sensitive (IS) non-diabetic subjects. Control group (C) consisted of 10 healthy, non-obese subjects. Insulin resistance was assessed by insulin-suppression test with somatostatin. Insulin secretion was measured as area under the curve AUC₁₂₀ during OGTT. In all subjects 24-hour blood pressure (BP) and heart rate (HR) monitoring was performed. Fasting blood samples for NA, A and DA levels were taken in supine position on a low-, normo- and high sodium diet. **Results:** Insulin resistance measured as steady-state glucose levels at the end of insulin-suppression test were in DM, IR, IS, and C groups: 214±7, 174±5, 105±9 and 63±3 mg/dl, respectively. Insulin secretion AUC₁₂₀ was: 78±11, 108±9, 76±8 and 46±5 mU/l/min, respectively. BP values registered in 24-hour record were within the normal range in all investigated groups, however in DM patients daytime diastolic pressure and MAP were significantly higher than in controls. Significantly higher HR (day and night time) was observed in DM group and tendency to higher HR was observed in IR and IS subjects. NA, A and DA levels were significantly lower in IR than in IS subjects. **Conclusions:** 1. Higher HR observed in DM patients seems not to be related to hyperinsulinaemia and may reflect a primary increase in sympathetic tone 2. Increased sympathetic tone can play a role in a pathogenesis of insulin resistance and rise of blood pressure. 3. Sympathetic activity should be checked by other methods than measurements of plasma catecholamines.

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EFFECTS OF OBESITY ON METABOLIC INDICES OF GLUCOSE DISPOSAL IN WOMEN WITH PRIOR GESTATIONAL DIABETES.

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Background and Aims: Obesity is the most prominent risk factor for type 2 diabetes. Thus, it is important to study its impact on glucose disposal and the interplay between insulin secretion and insulin sensitivity in women with former gestational diabetes (GDM), which often precedes type 2 diabetes. **Materials and Methods:** 60 GDM were studied by 75-g OGTT with frequent measurements (in a 180 min interval) of glucose, insulin, and C-peptide concentrations 3-4 months after delivery. According to their BMI (cut-off value: 27.5 kg/m²) GDM were divided into an obese (OB, N=28, BMI 31.4±0.8 kg/m²; basal glucose, insulin, C-peptide: 5.54±0.17 mmol/l, 73.2±7.2 pmol/l, 1037.7±182.7 pmol/l) and a normal-weight (NW, N=32, BMI 23.7±0.4 kg/m²; 4.81±0.06, 42.6±4.2, 534.9±35.7, p<0.02) subgroup. Total insulin secretion (TIS) and insulin sensitivity (OGIS) during the OGTT were derived from mathematical modeling analysis, overall glucose disappearance was described by the disposition index (DI=OGIS x deltaAUCins, with deltaAUCins the incremental OGTT-stimulated component of the insulin area under the curve). The ability of the B-cell to adapt to insulin resistance was estimated by the adaptation index (AI=OGIS x deltaTIS, with deltaTIS the incremental component of TIS). **Results:** Glucose and insulin levels were higher in OB (glucose AUC: 1.42±0.07 mol/l min vs 1.14±0.04, p=0.0006; insulin AUC: 65.7±7.1 nmol/l min vs 44.9±4.5, p=0.015) as was TIS (35.2±4.6 nmol/l vs 24.3±1.7, p=0.02); the latter was mostly due to an increased basal component (65.2±11.6 pmol/l/min vs 31.5±2.1, p=0.004), while the dynamic phase did not differ significantly. Despite markedly reduced insulin sensitivity (OGIS OB: 393±15 ml/min/m², NW: 448±12, p=0.006) overall glucose homeostasis was not significantly different as shown by similar metabolic indices DI and AI (DI: 20.3±2.1 nmol/m² in OB vs 16.2±1.3 in NW; AI: 9.3±1.3 nmol/min/m² in OB vs 8.1±0.5 in NW, p>0.09). **Conclusions:** In obese GDM, insulin secretion increases adequately for marked insulin resistance, avoiding a completely deranged glucose metabolism. However, B-cells cannot entirely compensate for prevailing hyperglycemia that, together with hyperinsulinemia, may increase the risk for manifestation of type 2 diabetes.

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Variants in the β -adrenergic receptor genes in obese children and adolescents.

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Background and Aims: β -adrenergic receptors play an important role in the regulation of lipolysis and thermogenesis by catecholamines. Genetic defects in expression or function of β 1-, β 2- and/or β 3-adrenergic receptors could affect energy homeostasis and predispose an individual towards the development of obesity. We therefore investigated the possible association of polymorphisms in the β -adrenergic receptor genes with early-onset obesity.

Materials and Methods: Genomic DNA was obtained from 294 extremely obese children and adolescents compared to 134 underweight control subjects. Genotyping was performed by PCR amplification with specific oligonucleotides, restriction enzyme digestion and agarose gel electrophoresis to assess allele and carrier frequencies of the following variants: Arg/Gly in codon 389 of the β 1-adrenergic receptor, Arg/Gly in codon 16 and Gln/Glu in codon 27 of the β 2-adrenergic receptor, Trp/Arg in codon 64 of the β 3-adrenergic receptor.

Results: The Gly389 allele in the β 1-adrenergic receptor was found with a frequency of 0.319 in obese and 0.328 in lean subjects (p=0.802). Gly16 in the β 2-adrenergic receptor was found with an allele frequency of 0.590 in obese and 0.611 in lean subjects (p=0.591). The Glu27 allele in the β 2-adrenergic receptor was found with a frequency of 0.380 in obese children and 0.420 in lean controls (p=0.298). The Arg64 allele in the β 3-adrenergic receptor was found with a frequency of 0.077 in obese and 0.065 in lean subjects (p=0.554). No significant differences were found for the individual genotype frequencies. Furthermore, no significant differences were found between obese and lean subjects regarding the distribution of individuals with variants in none, one, two or all three β -adrenergic receptors.

Conclusions: To our knowledge, this is the first study to assess the frequency of variants in all β -adrenergic receptors in cohorts of different body weight extremes. Neither did we find a significant association of a single polymorphism with obesity nor was there evidence for an additive or even synergistic effect of multiple variants within two or three adrenergic receptors in one individual. Therefore, our data make it rather unlikely that genetic variants in β -adrenergic receptors are involved in the pathogenesis of early-onset obesity.

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MUSCLE LIPID IN ADOLESCENT MALES: ASSOCIATIONS WITH ANTHROPOMETRY, INSULIN SENSITIVITY AND PHYSICAL ACTIVITY.

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Background and Aims: Insulin resistance of skeletal muscle is one of the factors implicated in the development of the 'Metabolic Syndrome', which encompasses obesity, diabetes and cardiovascular disease. Previous studies have shown that intramyocellular lipid (IMCL) levels as determined by 1H magnetic resonance spectroscopy (1H MRS) reflect insulin sensitivity determined by hyperinsulinaemic-euglycaemic clamp in the soleus muscle. However, people with Type 2 diabetes, whose muscles are resistant to insulin, have high IMCL levels, while highly-trained athletes, whose muscles are very sensitive to insulin, also have high IMCL levels (determined by biopsy). **Materials and Methods:** We took 22 males aged 16 to 21 years and compared their BMI, waist circumference, waist to hip ratio, sum of 5 skinfolds, physical activity hours per week, Peak VO2 on a treadmill, maximum heart rate, ventilatory threshold and homeostatic model assessment (HOMA) to their 1H MRS IMCL levels in the vastus lateralis and soleus muscles. Participants had BMI values between 17.9 and 30.1 kg/m², and VO2 Peak values between 40.7 and 62.4 ml/min/kg body weight. **Results:** HOMA (R²=0.736, p=0.000), sum of 5 skinfolds (R²=0.665, p=0.001), BMI (R²=0.507, p=0.016) and waist circumference (R²=0.414, p=0.044) were significantly positively correlated with m. vastus lateralis IMCL. No significant associations were found between these variables and IMCL content of the soleus muscle. In a stepwise multiple regression analysis, HOMA is the only significant predictor of m. vastus lateralis IMCL content. There were no significant associations between physical activity indicators and IMCL content of either muscle. **Conclusions:** The m.vastus lateralis IMCL content was significantly associated with insulin sensitivity as measured by HOMA, whereas the soleus muscle demonstrated no such association. This may be due to differing oxidative and muscle fibre-type characteristics between the two muscles. The results of this study would suggest that the vastus lateralis muscle is a more sensitive and more applicable muscle in which to study IMCL content with respect to insulin sensitivity, anthropometry and treadmill exercise. We conclude that in adolescent males, insulin sensitivity is the strongest predictor of vastus lateralis muscle IMCL content, and that physical fitness indicators (particularly aerobic fitness levels) are not influential.

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Insulin Resistance and Associated Conditions

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Insulin sensitivity towards glycemia and blood free fatty acids, as deduced from basal data or oral glucose tolerance test values, in various disease states
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Background and Aims: To measure insulin sensitivity towards glycemia and blood free fatty acids (FFA) in patients with various diseases.

Materials and Methods: Insulin Sensitivity Indices concerning the insulin effect on both glycemia and blood FFA, named ISI(gly) and ISI(ffa), were obtained by using the following formulae:

$ISI(gly) = 2 / [(INSp \times GLYp) + 1]$, and $ISI(ffa) = 2 / [(INSp \times FFAp) + 1]$, where $INSp$, $GLYp$ and $FFAp$ = insulinemic, glycemic and FFA areas (A) during OGTT (75 g glucose, suggested sampling at 0, 1 and 2 h) or basal values (B). Areas and basal levels are expressed by taking the mean normal value as 1, so that in normal subjects ISI(gly) and ISI(ffa) are always close to 1, with maximal variations between 0 and 2. **Results:** In normal subjects (n= 36), both indices were close to 1. In obese subjects (n= 30), ISI(ffa)-A was more severely reduced than ISI(gly)-A (values: 0.57 ± 0.04 and 0.46 ± 0.04 , respectively). A similar behavior was shown by ISI(ffa)-B and ISI(gly)-B. In obese-diabetic patients (n= 14) all four indices were markedly lowered (mean values between 0.40-0.44). In obese diabetic patients, treatment with metformin (1.7g/day, n= 12) for 10 days improved insulin sensitivity of blood glucose [ISI(gly)-A by 45% and ISI(gly)-B by 54%] but not that of blood FFA. Hypertensive-obese subjects (n= 13) compared to matched obese without hypertension (n=15) showed marked reduction in both ISI(gly) and ISI(ffa) (mean values between 0.54-0.65), with a trend to prevalent impairment of the latter. For all changes, the p' range was 0.05-0.001.

Conclusions: Our Indices are simple to perform and are carried out under physiological conditions, thus being suitable for clinical and epidemiological studies. They allow to compare the behavior of insulin sensitivity of glycemia and that of blood FFA in various disease states.

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AGING, NOT INSULIN RESISTANCE, IS THE MAJOR DETERMINANTS OF AORTIC PULSE WAVE VELOCITY IN NORMOTENSIVE NONDIABETIC SUBJECTS

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Background and aims: Previous studies have shown that aortic stiffness, expressed by higher values of pulse wave velocity (PWV), is associated with aging and diabetes. However, the role of insulin resistance in mediated vascular sclerotic change in nondiabetic subjects remains largely unknown.

Materials and methods: Insulin resistance, assessed by insulin suppression test, and aortic distensibility, expressed by aortic to femoral PWV, were determined in 24 nondiabetic, normotensive subjects with (mean \pm SEM) age 43 ± 2 years and body mass index (BMI) 23.6 ± 0.6 Kgs/m². **Results:** Values of PWV showed a positive correlation with age and degree of insulin resistance, expressed as steady state plasma glucose (SSPG) levels, but not with BMI, blood pressure, fasting glucose, insulin or lipid concentrations. Multiple regression analysis showed that age (P=0.033) was independently associated with values of PWV but SSPG values (P=0.356) was not. When the SSPG concentrations were divided into three tertiles, the PWV values (9.8 ± 0.8 versus 11.3 ± 1.1 versus 10.0 ± 0.8 m/s, p=0.452) were not different across these three subgroups. **Conclusion:** Age, but not insulin resistance, is the major determinant of aortic distensibility in a group of normotensive, nondiabetic subjects.

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RATES OF INSULIN SECRETION AND PERIPHERAL GLUCOSE DISPOSAL AFTER A MIXED MEAL IN PATIENTS WITH HYPERTHYROIDISM

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Background and Aims: Glucose intolerance is common in hyperthyroidism but the mechanisms are unclear. Insulin secretion has been found increased or decreased. Increased glucose production due to insulin resistance is firmly established but the effects of insulin on peripheral glucose utilization are variable. The aim was to assess in hyperthyroid patients (HYPER) postprandial (a) insulin secretion and (b) insulin effects on glucose disposal (GFLUX) in muscle (M) and adipose tissue (AD). **Patients and Methods:** A standard mixed meal was given to 11 HYPER (age 36 ± 4 yrs, BMI 24 ± 1) and 6 controls (C, age 41 ± 5 yrs, BMI 22 ± 1). Plasma samples for measurements of glucose (G) and insulin (I) were taken for 360min from veins (V) draining the anterior abdominal subcutaneous AD depot and the forearm M, and from an arterialized hand vein (A). With each blood sample, blood flow (BF) was measured in AD (with ¹³³Xe) and M (with strain-gauge plethysmography). Calculations: $GFLUX = [G(A-V)] \times [BF]$; Clearance of G (GCL) = $[GFLUX/G \text{ in A}]$. Postprandial changes are presented as area under curve (0-360 min). **Results:** In HYPER vs C: (1) Arterialized G was not different (368 ± 54 vs 345 ± 63 mM/min), whereas I was increased (81 ± 7 vs 56 ± 9 nM/min, p<0.05). (2) BF was increased in M (2968 ± 323 vs 2000 ± 201 ml/100_{ct}tissue, p<0.05) and AD (1728 ± 204 vs 1133 ± 131 ml/100_{ct}tissue, p<0.05). (3) GFLUX was not different in M (630 ± 140 vs 1055 ± 189 μ mol/100_{ct}tissue) and AD (413 ± 94 vs 290 ± 57 μ mol/100_{ct}tissue). (4) GCL was decreased in M (107 ± 25 vs 193 ± 30 ml/100_{ct}tissue, p<0.05) whereas the respective values in AD were not different (83 ± 20 vs 51 ± 10 ml/100_{ct}tissue). **Conclusions:** In hyperthyroidism, in the postprandial state: (1) Insulin secretion is increased. (2) Insulin stimulation of glucose disposal in muscle is impaired; this defect is corrected, at least in part, by increases in blood flow.

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OBSTRUCTIVE SLEEP APNEA SYNDROME 'PER SE' IMPAIRS INSULIN SENSITIVITY

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Background and Aims: Visceral obesity, along with the other metabolic abnormalities of the 'insulin resistance' or 'metabolic' syndrome, is associated with increased risk of cardiovascular diseases (CVD). Obstructive Sleep Apnea Syndrome (OSAS) is commonly associated with obesity and is connoted by specific worsening in endocrine and metabolic abnormalities (such as GH/IGF-I, HPA, thyroid and gonadal axis as well as glucose metabolism). In fact, further increase in cerebro- and cardio-vascular risk in patients with OSAS is indicated by retrospective studies and could be independent of overweight. Studies of the relationship between insulin sensitivity and OSAS are scanty. **Materials and Methods:** The insulin resistance index HOMA-IR (Homeostasis Model Assessment) was determined in 25 male obese patients with OSAS (mean \pm ESM, age: 55.7 ± 2.04 years; BMI: 35.8 ± 1.07 kg/m²; Apnea-Hypopnea Index, AHI: 41.8 ± 5.44), in 24 male patients with simple obesity (OB, age: 49.7 ± 2.02 years; BMI: 39.2 ± 1.6 kg/m²; AHI: <5) and in 153 normal subjects (NS, 153; age: 42.8 ± 1.32 years; BMI: 23.3 ± 0.32 kg/m²). In all obese patients nocturnal polysomnography was also performed to determine the Apnea-Hypopnea Index (normal value: < 5). The statistical analysis was performed by means of Analysis of Covariance (ANCOVA) with age, BMI and Waist-to-Hip Ratio (WHR) as covariate variables, and Neuman-Keuls test where appropriate. **Results:** HOMA estimation of insulin resistance was different among the three groups (ANCOVA: p<0.00006). HOMA-IR values in OSAS were greater than those in OB (8.90 ± 0.76 vs 5.09 ± 0.47 , p<0.00001) which, in turn, were higher than in NS (5.09 ± 0.47 vs 3.18 ± 0.15 , p<0.0063). It has to be emphasized that overweight in OSAS was higher than in OB (p<0.004). **Conclusions:** Obese patients with obstructive sleep apnea are more insulin resistant than patients with simple obesity. Thus OSAS further impairs insulin sensitivity in obesity independently of the degree of adiposity. The worsening in insulin sensitivity in OSAS patients could reflect the hypoxic state and would account for the increased vascular risk in this condition.

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INSULIN RESISTANCE IS A PROMINENT FEATURE OF CHRONIC HEART FAILURE INDEPENDENT OF THE UNDERLYING AETIOLOGY.

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Background and Aims: To investigate abnormalities regarding insulin sensitivity in patients with chronic heart failure (CHF) and their relation to the underlying aetiology. **Materials and Methods:** Twenty male patients (group A) with stable CHF of more than 6-mo duration (A1: 10 due to ischemic heart disease, A2: 10 due to non-ischemic cardiomyopathy, comparable according to EF and functional class-NYHA) and ten male control individuals (group B) were recruited. Insulin sensitivity was assessed by euglycaemic hyperinsulinaemic clamp technique (40mU/m²/min) by estimating the average glucose infusion rate (M value: umol/Kg/min) and insulin sensitivity index (ISI) during the last hour of the test. Patients with diabetes mellitus, impaired glucose tolerance (after oral glucose tolerance test), impaired fasting glycaemia, hepatic, thyroid and renal dysfunction were excluded from the study. **Results:** The three groups were comparable according to age, BMI and WHR. Patients with CHF had higher insulin levels (137.1 ± 18.3 pmol/L vs. 75.1 ± 6.7 pmol/L, p = 0.022). Insulin sensitivity was significantly impaired in patients with CHF compared to controls [M value: 23.01 ± 1.2 umol/Kg/min vs. 34.08 ± 1.7 umol/Kg/min, p < 0.001, ISI: 29.8 ± 2.9 vs. 48.28 ± 2.74, p < 0.001]. Regarding the underlying aetiology both ischemic and non-ischemic CHF were associated with impaired insulin sensitivity compared to controls (M-value: ANOVA : p < 0.001; A1 vs. B: p < 0.001; A2 vs. B: p = 0.001; and ISI: p < 0.01; A1 vs. B: p < 0.001; A2 vs. B: p = 0.01) with ischemic CHF patients significantly more insulin resistant compared to non-ischemic (A1 vs A2: p = 0.027; A1 vs A2: p < 0.01, for M-value and ISI respectively). Regression analysis revealed that the presence of both ischemic (SC = - 0.894) and non-ischemic CHF (SC = - 0.522) and fasting triglycerides (SC = - 0.301) were independent predictors of impaired insulin sensitivity (adjusted R² = 0.711, p < 0.001). **Conclusions:** Insulin resistance is a prominent feature in patients with CHF. Although ischemic heart disease is associated with more marked insulin resistance, the presence of chronic heart failure, independent of the underlying aetiology, is associated with impairment of insulin-mediated glucose disposal.

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Development of diabetes in hepatitis C virus infected patients: The role of insulin resistance and autoimmunity

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Background and Aim: The rate of diabetes increases in patients with Hepatitis C virus (HCV) chronic infection. Although, autoimmune diseases are induced by HCV infection, this relationship remains controversial for diabetes yet. The aim of the study was to evaluate the development of diabetes in HCV infected patients whether due to autoimmune Beta cell damage or insulin resistance seen in hepatocellular insufficiency.

Material and Methods: The prevalence of islet cell autoantibodies (Glutamic acid decarboxylase antibodies-GADAs and Islet Cell Antibodies-ICAs) were assessed in 63 nonselected HCV infected patients (47 diabetic, 16 nondiabetic) and in 24 age, sex and BMI matched control subjects (nonHCV infected, diabetic). Beta cell secretion capacity (HOMA-B) and insulin sensitivity index (HOMA-S) were performed by using Homeostasis Model Assessment (HOMA).

Results: GADAs were detected in 3 of 47 (6 %) HCV (+) diabetic patients, 1 of 16 (6 %) HCV (+) nondiabetic patients, and 2 of 24 (8 %) in HCV (-) diabetic control patients (p=NS). ICAs were positive in 3 of 47 (6 %) HCV infected diabetic patients and 1 of 16 (6 %) HCV infected nondiabetic patients on the other hand negative in HCV (-) diabetic control patients (p=NS). HOMA-B of HCV (+) nondiabetic patients were found significantly higher than HCV (+) and HCV (-) diabetic patients (331 ± 128% vs 210 ± 97 %, p<0.001, and 331 ± 128 % vs 54±47 %, p<0.001, respectively). There was also significant difference between HCV (+) and HCV (-) diabetic patients (p<0.001). In addition, HOMA-S results showed no difference between HCV (+) nondiabetic and diabetic patients (12 ± 7 % and 12 ± 6 %, p=NS), but significantly higher values in HCV (-) diabetic patients (41 ± 37 % vs 12 ± 7 % and 12 ± 6 %, p<0.001 for both).

Conclusion: Our results suggest that Beta cell autoimmunity is not associated with HCV infection. So the increased diabetes mellitus prevalence among HCV infected patients could be mediated by insulin resistance generally seen in hepatocellular insufficiency.

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Insulin resistance and hyperandrogenemia. The impact of metformin therapy

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Background and Aims: Hyperandrogenemia and menstrual disorders are increasingly recognized to depend on insulin resistance. Here we prospectively investigated women referred to our Department for menstrual disorders or hirsutism as to their frequency of insulin resistance and tested the effectiveness of metformin therapy under these conditions.

Materials and Methods: 55 patients were included in the study (age 31.5±7.4 yrs; BMI 33.8±7.0 kg/m²). Insulin resistance was tested using the HOMA/CIGMA model. Serum levels of insulin, LH/FSH, 17-OH progesterone, total and free testosterone and androstendion were measured using commercially available RIA's. 16 subjects received metformin therapy (850 mg trice daily) for a minimum of 6 months; 8 subjects served as controls.

Results: According to HOMA/CIGMA 25 subjects were insulin resistant with an insulin sensitivity of < 70%. The BMI of these subjects was >30 kg/m² in 65 %, 25-30 kg/m² in 9% but in 26 % lower than 25 kg/m². Metformin significantly reduced the LH/FSH ratio, insulin levels, 17-OH progesterone, androstendione, total and free testosterone (p<0.02 - 0.001) whereas control subjects did not change. Menstrual cycles improved in 14 of the subjects under metformin whereas 2 of 8 controls showed some improvement.

Conclusions: App. 45 % of our patients presenting with hirsutism and/or menstrual disorders were insulin resistant according to HOMA/CIGMA testing and 25 % of these (i.e. app. 11%) had normal body weight. Insulin resistance was functionally important as shown by the successful treatment by metformin. These data support the close link between PCO, hirsutism and menstrual disorders with insulin resistance and indicates that these symptoms serve as a marker for insulin resistance even in normal weight subjects.

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Peripheral Glucose Metabolism in Patients with Psoriasis

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Background and Aims: The present study was designed to determine the effect of psoriasis on peripheral glucose metabolism.

Materials and Methods: Ten non-obese patients with psoriasis vulgaris (Psoriasis Area and Severity Index of 10.1 ± 2.1 scores) and 11 healthy non-obese controls matched for age and BMI were studied after an overnight fast (12-14 h) and for 3 hours after ingestion of 75 g of glucose. Peripheral glucose metabolism was evaluated by the forearm technique to estimate muscle exchange of substrate combined with indirect calorimetry. Arterial and venous blood samples were collected by deep vein catheterization of the dominant forearm and the contralateral brachial artery under basal conditions and at 30, 60, 120 and 180 minutes after ingestion of 75 g of glucose for the determination of glucose, insulin, free fatty acids, and O₂ and CO₂ levels. Arteriovenous differences associated with blood flow measurements by capacitance plethysmography allowed the quantification of muscular metabolic flows, and carbohydrate and lipid oxidation rates were quantified by indirect calorimetry. Data were analyzed statistically by the Mann-Whitney test (p<0.05).

Results: Increased serum insulin (71 ± 8 vs. 49 ± 8 pmol/L) and normal glucose levels during fasting were observed in patients with psoriasis vulgaris compared to controls. After the glucose overload, blood insulin and glucose concentrations, and glucose uptake and oxidation did not differ significantly between groups. However, the patient group presented a delay of insulin secretion, with the maximum increase occurring at 120 minutes. Muscular glucose utilization in non-oxidative metabolism was also significantly decreased in psoriatic patients when compared to controls (3.9 ± 0.8 vs. 6.0 ± 0.6 mmol/100 ml forearm-l x 3 h-l). Both free fatty acid levels and lipid oxidation rates were similar in normal subjects and psoriatic patients and declined in a similar fashion after glucose ingestion, suggesting an adequate insulin sensitivity of adipose tissue.

Conclusions: These data may suggest a preliminary state of muscular insulin resistance in patients with psoriasis manifested by impaired non-oxidative metabolism and delayed insulin response to glucose overload. The increased fasting insulin levels observed in the psoriatic group may indicate that hepatic insulin resistance also plays a role in the insulin resistance found in this disease.

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EVALUATION OF INSULIN SENSITIVITY AND BETA CELL FUNCTION IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

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Polycystic ovary syndrome (PCOS) is often connected with decreased insulin sensitivity (IS) and disturbances of β -cell function (β F). The aim of our study was to evaluate the IS and the β F in a broad spectrum of PCOS women (BMI 18.7-47.4 kg/m²) using fasting, oGTT and insulin-tolerance test (ITT) derived indices, to compare them with healthy eumenorrhoeic women (C) and to find the best predictive variables of the impaired insulin action in the PCOS patients.

Methods: 22 healthy control women with negative family history of PCOS and diabetes, aged 28 \pm 6.8 yrs, and 54 women with PCOS, aged 25 \pm 6.4 yrs (mean \pm SD) were studied. In the early follicular phase of menstrual cycle 3-hour oGTT with estimation of blood glucose (G), insulin (I), C-peptide (Cp) and proinsulin (PI) in 0, 30, 60, 120, 180 min., 15-min ITT (K_{ITT} , G_{minITT} , AUC- G_{ITT}) and anthropometric parameters were determined. After the adjustment to BMI, ANCOVA was performed (Statgraphics Plus 3.0). In addition, logistic regression and ROC curves were used (NCSS 2000). **Results:** In PCOS, significantly higher waist/hip ratio (WHR, $p < 0.0001$), G_{180} ($p < 0.05$), I_{30} ($p < 0.05$), I_{120} ($p < 0.05$), $\Delta I/\Delta G_{30-0}$ ($p < 0.001$), Cp_0 ($p < 0.001$), Cp_{180} ($p < 0.05$), K_{ITT} ($p < 0.05$), the G_{minITT} ($p < 0.01$) and AUC- G_{ITT} ($p < 0.0001$) were found. The best discrimination of PCOS from C was achieved using combination of WHR and G_{minITT} . The final model correctly classified 91.7% of patients ($r = 0.713$, $p < 0.0001$). The physiological hyperbolic relationship was found in all tested combinations of IS and β F indices. The PCOS differed most pronouncedly from C using the combination of $\Delta I/\Delta G_{30-0}$ and AUC- G_{ITT} ($p < 0.0001$). **Conclusions:** In our PCOS patients, the increased β F was found. The impaired insulin sensitivity was evident especially after insulin stimulation during the ITT. The best discrimination of PCOS from healthy C was achieved using combination of WHR and ITT.

Supported by IGA 4847-3, IGA 5395-5 MII CR, MII 000000023761.

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The Effect Of Acute Hyperinsulinaemia On Erythrocyte Membrane Ion Transport And Relation To Insulin Action In Offspring Of Hypertensive Parents

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Background and Aims: Several erythrocyte membrane ion transport abnormalities and insulin resistance (IR) are exhibited in patients with essential hypertension. Aims: a) To assess erythrocyte membrane ion transport in offspring of hypertensive parents and healthy controls, b) to test in vivo effect of acute hyperinsulinaemia and c) to evaluate relation to IR.

Materials and Methods: We measured the activities of Na⁺-K⁺ pump, Na⁺-K⁺ cotransport and Na⁺-Li⁺ countertransport and passive membrane permeability (leak) for Na⁺, Rb⁺ and Li⁺ in offspring of hypertensive parents ($n = 12$; OHP) and healthy controls ($n = 14$; C). Ion transport was assessed before and in fifth hour of hyperinsulinaemic euglycaemic clamp (HEC) lasting 10 hours (insulinaemia 75 μ U/ml) and compared to that found under isoinsulinaemic isovolumic conditions. Insulin action was measured as glucose disposal (M) and insulin sensitivity index (M/I) in period 280-300 min of HEC.

Results: M/I was significantly lower in OHP compared to C (0.12 ± 0.07 vs 0.20 ± 0.09 mg. kg⁻¹. min⁻¹. μ U⁻¹. ml; $p < 0.05$). Elevated Na⁺-Li⁺ countertransport (0.080 ± 0.004 vs 0.068 ± 0.003 mmol.l⁻¹.h⁻¹; $p < 0.05$), Li⁺ leak (0.106 ± 0.004 vs 0.093 ± 0.003 mmol.l⁻¹.h⁻¹; $p < 0.05$; $p < 0.05$) and Rb⁺ leak (0.160 ± 0.014 vs 0.120 ± 0.007 mmol.l⁻¹.h⁻¹; $p < 0.05$) were found in OHP, but there were no significant differences in other transport activities. M correlated in all patients with Li⁺ leak after HEC ($r = -0.424$; $p < 0.05$), in OHP with Na⁺ leak ($r = -0.727$; $p < 0.05$) before HEC and in C with Li⁺ leak ($r = -0.736$; $p < 0.01$) after HEC. Acute hyperinsulinaemia did not modify significantly any of the investigated transport parameters.

Conclusions: OHP displayed higher insulin resistance, enhanced activity of Na⁺-Li⁺ countertransport and augmented passive membrane permeability for monovalent cations. Negative correlation was noticed between insulin action and membrane leaks. Acute hyperinsulinaemia did not modify any RBC ion transport. (Supported by grant IGA MZ CR:NB/6682-3)

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Effects of hyperinsulinemia with euglycemic and eu-aminoacidemic conditions on fibrinogen synthesis in type 2 Diabetes Mellitus (T2DM).

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Backgrounds and Aims: Fibrinogen is a strong cardiovascular risk factor in the general population, and increased fibrinogen plasma concentrations have been reported in T2DM. However, the mechanisms leading to hyperfibrinogenemia in T2DM are not known. In normal subjects insulin (hyperinsulinemic, hypo-aminoacidemic clamp) inhibits fibrinogen fractional synthetic rate (FSR). However, the effect of insulin on fibrinogen synthesis in T2DM is not known.

Materials and Methods: To address this question, fibrinogen FSR was determined in 8 male T2DM patients and 7 non diabetic matched Controls (C) using leucine isotope precursor-product relationships, in the basal state and following a 4-hr euglycemic-hyperinsulinemic (100 uU/ml) clamp. Amino acid concentrations were maintained at the basal level in the hyperinsulinemic state using i.v. amino acid infusions.

Results: In the basal state, fibrinogen concentrations in the T2DM (363 ± 42 mg/dl) were greater ($p < 0.05$) than in C (258 ± 17). Fibrinogen FSR was not different between T2DM (24.4 ± 3.2 percent/day) and C (21.1 ± 2.2). During the clamp, FSR increased ($p < 0.01$) to 35.6 ± 4.4 percent/day in T2DM whereas it did not change in C (to 22.6 ± 2.6). A negative correlation was found between insulin-mediated glucose disposal and the increase in fibrinogen FSR ($r = -0.58$, $p < 0.05$).

Conclusions: 1) Physiologic hyperinsulinemia with euglycemic and eu-aminoacidemic conditions stimulates fibrinogen FSR in T2DM. 2) In control subjects, euglycemic hyperinsulinemia with eu-aminoacidemia does not decrease fibrinogen FSR, as opposed to previous clamp studies with hypo-aminoacidemia. 3) The increase in fibrinogen FSR may be associated with insulin resistance. 4) Plasma amino acids may modulate the effects of insulin on fibrinogen FSR.

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Improving Insulin Sensitivity

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Effects of short-duration skeletal muscle exercise on glycogen synthesis during hyperinsulinemia. A study using 13C-Magnetic Resonance Spectroscopy
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Background and Aims: Both exercise and insulin stimulate glucose uptake in skeletal muscle, by translocation of GLUT 4 protein to the plasma membrane. Little is known about the additional effect of exercise upon insulin stimulated glucose uptake. 13C-MRS enables continuous, non-invasive measurement of glycogen synthesis in skeletal muscle in humans. In this study, we measured the effect of a short bout of exercise on insulin-induced glycogen synthesis and blood flow in skeletal muscle.

Materials and Methods: Five healthy subjects (age 20.2 ± 1.8 yrs, BMI 21.3 ± 1.4 kg/m²) underwent a euglycemic hyperinsulinemic (430 pM/m²/min) clamp, using 1-13C-glucose labeled infusion for 150 min, with simultaneous measurement of glycogen in skeletal muscle. Measurements were performed on a 1.5T MRI (Siemens Vision, Erlangen), adapted to measure 13Carbon, with a home-build quadrature 1H, circular 13C coil (Settings: Tr 180 ms, continuous wave decoupling turned on during 60 ms at 30 W. Number of scans 5000 and 2500. Glycogen, glucose and creatin levels were determined at the calf muscle. Glycogen synthesis rate, determined as the increase in glycogen signal in time was corrected for plasma 1-13C-glucose enrichment. Also C1-glucose anomer signals were determined. After baseline measurements, acute local exercise in the calf muscle at 50% maximum voluntary contraction was performed by one leg toe lifting for 1 min, twice. MRS measurements were continued for another 60 min. On a separate day, the whole experiment was repeated, with measurement of blood flow at both legs and one arm before and after exercise.

Results: Baseline insulin-stimulated glycogen synthesis was 0.332 ± 0.08 AU/min; exercise increased glycogen synthesis substantially to 1.013 ± 0.286 AU/min ($p < 0.01$), relative increase 3.09 ± 0.92 . After exercise, calf blood flow in the exercised leg increased from 1.4 ± 0.3 to 18.7 ± 5.3 mL/dL/min, but blood flow returned to 2.9 ± 0.6 within 30 min, while the increase in glycogen synthesis was stable and ongoing. The C1-glucose showed a significant increase after exercise from 40.6 ± 17.3 to 59.8 ± 5.8 g ($p < 0.05$), a relative increase of 1.5.

Conclusions: In humans, a very short bout of exercise during hyperinsulinemia, substantially increases glycogen synthesis rate. Although exercise was also a strong stimulus for vasodilatation (explaining the increase in glucose content), the lack of a temporal relationship between the increase in blood flow and the increase in glycogen synthesis argues against a direct causal effect.

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The Use of CSA Accelerometers To Investigate the Relationship of Physical Activity to Insulin Resistance in Five-Year-Old Children
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Background and Aims: Low levels of physical activity (PA) have been blamed for increasing childhood obesity, insulin resistance (IR) and its metabolic complications, which include type 2 diabetes. Electronic accelerometers are revolutionising our ability to profile children's physical activity.

Materials and Methods: EarlyBird is a prospective cohort study monitoring the physiognomy, health and lifestyle of 300 healthy school entrants (mean age 4.8 years). Each has worn a CSA accelerometer (validation reported elsewhere) for seven consecutive days to monitor activity both during a school week and at the weekend. PA is expressed in arbitrary units, resolved into low, medium and high intensity activity. IR was measured by homeostasis model assessment (HOMA) and visceral fat by ultrasound.

Results: (n=100): Boys totalled more daily PA than girls, both during the school week and at weekends ($p < 0.05$), and the difference was almost wholly accounted for by high intensity activity. There was a weak (positive) relationship between current weight and total PA (max $r = 0.17$). Significantly more ($p < 0.05$) PA was recorded on weekend days (5.11×10^4 5 units) than on school days (4.73×10^4 5) in both sexes. The range of mean daily counts rose from 2.2-fold (2.91×10^4 5- 6.50×10^4 5) on school days, to 4.6-fold (1.94×10^4 5- 8.84×10^4 5) at the weekend. Most importantly, there was a strong relationship ($r = 0.65$, $p < 0.001$) between a child's PA on a school day and on a weekend day, making it possible to identify the 'physically inactive phenotype'. Overall, there was no relationship at 5y between total PA and IR. In boys only, there was an inverse relationship between high intensity PA and visceral fat ($r = -0.40$, $p < 0.05$) and a direct relationship between IR and visceral fat ($r = 0.33$, $p < 0.05$).

Conclusions: 1) Five-year-old school children are generally more active at weekends than during the school week. 2) Differences between individual children are striking. An 'inactive phenotype' can be identified, even at 5y - the child who is inactive throughout the school week as well as at weekends. 3) IR is related to body weight at 5y only in girls (reported elsewhere), an observation which could be explained by the greater amount of high intensity PA recorded among boys.

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Role of physical activity, resting energy expenditure and diet-induced thermogenesis in weight loss achievement.

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Background and aims: Obesity results from an imbalance between increased energy intake and reduced energy expenditure. A low resting energy expenditure (REE) has been shown to predict weight gain and obesity. Several cross-sectional studies have reported a blunted diet-induced thermogenesis (DIT) in obese people, leading to the hypothesis that defective thermogenesis may contribute to the development of obesity. Furthermore, decreased fat oxidation has been documented in reduced-obese subjects, but in those studies only fasting RQ was measured; a definitive assessment of the ability to oxidize fat in reduced-obese requires measuring fat oxidation over 24 hours.

Materials and methods: We measured REE, DIT, physical activity (PA), substrate oxidation and EE during moderate exercise with the use of a respiratory chamber, in 20 morbidly obese subjects (BMI > 40 kg/m²) undergoing either bariatric surgery (bilio-pancreatic diversion) or low-calorie diet.

Results: Pre-Post weight loss differences in DIT were significantly higher ($P = 0.034$) in the BPD group (0.15 ± 0.05) compared to the dieting group (-0.03 ± 0.05). Furthermore, all patients showed a significantly ($P = 0.003$) higher average rate of carbohydrate oxidation after weight loss (Pre-Post $= -0.12 \pm 0.03$). Furthermore, weight loss was also explained by the increased physical activity, as resulted from regression analysis ($P = 0.0165$).

Conclusions: The present study provides no evidence to support the hypothesis that a lower than expected REE is an inescapable consequence of weight loss. Although we cannot exclude the possibility that there is an increased energy efficiency after weight loss in some subjects, the results we have obtained suggest that many people have a normal REE after weight loss. Comparing BPD patients and dietary-restricted patients it is likely that many subjects who fail to maintain weight loss do so primarily because they are not able to maintain dietary and physical activity patterns that are different from those that prevailed in their overweight state.

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COMPARISON OF THE EFFECTS OF EXERCISE AND INSULIN ON MUSCLE GLUCOSE UPTAKE AND PERFUSION IN TYPE 2 DIABETES

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Background and aims: Insulin resistance is a pivotal underlying metabolic abnormality in most patients with type 2 diabetes, but the mechanism of insulin resistance in humans is not fully understood. We have investigated whether insulin and exercise stimulated muscle glucose uptake and perfusion in patients with type 2 diabetes are decreased.

Materials and Methods: For this purpose, muscle glucose uptake ([18F]-FDG), blood flow ([15O]-H₂O) and oxygen consumption ([15O]-O₂) were measured with positron emission tomography (PET) in 8 patients with dietary treated type 2 diabetes (age 58 ± 2 years; BMI 32 ± 1 kg/m²) and 9 healthy age-matched subjects during euglycemic hyperinsulinemic conditions (insulin infusion rate 1 mU/kg*min) and one legged isometric exercise (anteromedial muscle compartment, 10 % of maximal power).

Results: Muscle oxygen consumption was ~15-fold higher in the exercising muscle compared to the resting contralateral muscle and similar during exercise in both groups. Insulin stimulated muscle glucose uptake was 36 % lower in the patients with type 2 diabetes (14 ± 1 μmol/kg muscle*min) than in the healthy subjects (22 ± 3 , $p < 0.05$). Furthermore, exercise stimulated glucose uptake to a lower extent in the diabetic group than in healthy subjects (21 ± 6 vs 57 ± 14 μmol/kg muscle*min, $p < 0.05$). Blood flow rates in resting muscle was not different between the two groups (27 ± 2 vs 36 ± 9 mL/kg muscle*min, patients with type 2 diabetes vs healthy subjects, NS). However, in exercising muscle healthy subjects had higher blood flow rates than patients with type 2 diabetes (136 ± 11 vs 189 ± 20 mL/kg muscle*min, $p < 0.05$).

Conclusions: These results demonstrate that the response to exercise is impaired in type 2 diabetes. This is caused by both a decreased muscle perfusion and a decreased glucose uptake.

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TEMPORAL CHANGES IN METABOLIC FITNESS WITH MODEST WEIGHT LOSS.

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Background and Aims: Current guidelines for obesity management emphasise the improvements in metabolic fitness associated with weight losses of 5-10% initial weight. However most of the data is derived from periods of acute weight loss and may not represent the true effect in the longer term. **Methods:** 57 overweight women (Age 43.7 ± 9.1 yrs, mean BMI 31.7 kg/m^2 , range $27.2-38.5 \text{ kg/m}^2$) with no other significant medical history, entered a milk-based low energy weight loss programme for 12 weeks and were then monitored without further intervention until 52 weeks. Weight, fasting plasma insulin, lipids and blood pressure were measured at 0, 12, 24 and 52 weeks. **Results:** The mean weight change in sequential periods was -11.6% ($p < 0.0001$), $+1.1\%$ ($p = 0.02$) and $+5.2\%$ ($p < 0.0001$). The change from baseline at one year being -6.0% ($p < 0.0001$). The table shows the baseline and mean change in metabolic parameters at 12 and 52 weeks (paired t-test).

Variable	units	Week 0	Week 12	p value	Week 52	p value
Weight	kg	85.9	-9.93	<0.0001	-5.16	<0.0001
Insulin*	pmol/l	51.9	-15.2	<0.0001	-3.8	0.20
Total Chol	mmol/l	6.23	-0.37	0.0003	0.18	0.21
LDL Chol	mmol/l	4.30	-0.29	0.011	0.066	0.63
HDL Chol	mmol/l	1.30	-0.01	0.70	0.061	0.27
Triglyceride*	mmol/l	1.38	-0.17	0.050	0.15	0.049
Systolic BP	mmHg	129	-7.2	0.0062	-6.8	0.0006
Diastolic BP	mmHg	86	-6.9	<0.0001	-5.5	0.0002

* Variable was logged for analysis. (Chol = Cholesterol)

After acute weight loss, there are significant improvements in metabolic fitness. However despite maintenance of more than 5% weight loss at one year only improvements in blood pressure remain significant. **CONCLUSIONS:** This study highlights the need for maintenance programmes to consolidate improvements in health in this group of modestly obese otherwise healthy women.

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EUGLYCEMIA IMPROVES HYPERGLYCEMIA-INDUCED INSULIN RESISTANCE IN PATIENTS WITH UNCONTROLLED TYPE 2 DIABETES

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Background and Aims: Chronic hyperglycemia causes insulin secretion defects and aggravates insulin resistance. This so-called glucose toxicity is reversible by any therapy achieving euglycemia. The duration of treatment, required for ameliorating hyperglycemia-induced insulin resistance is unknown in diabetic patients who are insufficiently controlled despite large doses of s.c. insulin (and thus are severely insulin resistant). This category of patients represents a true clinical problem. We investigated whether a period of euglycemia obtained by i.v. insulin treatment, followed by continuous subcutaneous insulin infusion (CSII) with a short-acting insulin analogue, could improve metabolic control and insulin sensitivity in patients with uncontrolled diabetes type 2, despite treatment. **Materials and Methods:** 8 Patients with type 2 diabetes (F:M=6:2, age 53 ± 13 yrs, BMI $38 \pm 6 \text{ kg m}^{-2}$), who were in bad metabolic control despite s.c. insulin treatment with a four-dose regimen (insulin dose $1.92 \pm 0.65 \text{ U/kg/day}$, HbA1c $12.0 \pm 1.7\%$), were admitted for 37 \pm 12 days and treated with i.v. insulin for 31 \pm 10 days, followed by CSII, using insulin Lispro. They also received education, diet and exercise instructions and metformin treatment. Blood glucose (BG) was measured 4 times daily. Insulin dose was individually titrated. Glycemic treatment goals were fasting and premeal BG $4.0-6.5 \text{ mmol/L}$. Before and after 28 \pm 6 days, a hyperinsulinemic euglycemic clamp (insulin $120 \text{ U m}^{-2} \text{ min}^{-1}$) was performed. After discharge they were followed frequently in the outpatient department. **Results:** After 15 \pm 6 days of treatment, patients were euglycemic and remained euglycemic while admitted. Insulin dose required to remain euglycemic decreased from 1.7 ± 0.9 to $1.1 \pm 0.6 \text{ U/kg/day}$ ($p < 0.005$). This period of euglycemia improved insulin sensitivity substantially: whole body glucose uptake, measured with the clamp increased from 12.7 ± 5.6 to $22.4 \pm 8.8 \text{ micromol/kg/min}$ ($p < 0.0005$). HbA1c decreased from 12.0 ± 1.7 to $8.6 \pm 1.1\%$ ($p < 0.0001$) after 28 days, to $7.3 \pm 0.8\%$ after 3 months ($n=8$), to $7.3 \pm 0.6\%$ after 6 months ($n=6$) and to $7.8 \pm 1.1\%$ after 1 year ($n=6$). Lipid levels improved as well. Mean weight did not change after 3 months and after 1 year. **Conclusions:** A period of strict metabolic control achieved by i.v. insulin followed by CSII using insulin analogues ameliorates the chronic hyperglycemia-induced insulin resistance. Remarkably, this improved metabolic control in our patients was sustained up to at least one year.

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Transjugular intrahepatic portosystemic shunt elevates insulinemia without any influence on insulin resistance

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Background and aims: Liver cirrhosis (LC) is characterized by insulin resistance (IR) and hyperinsulinemia (HI). Both, a higher insulin secretion rate and a reduced insulin hepatic clearance (IHC), contribute to HI. A decrease in IHC may be due either to hepatocellular dysfunction or to portosystemic shunting. TIPS (transjugular intrahepatic portosystemic shunt) takes an important part in the treatment of portal hypertension in patients with LC and presents a model situation for a sudden change in portal pressure. Aim of study was to find the answer to the question: Is HI and IR influenced by TIPS?

Materials and methods: Our study group consisted of 22 patients with LC (13 diabetics and 12 without DM). We analyzed insulin and C-peptide levels in peripheral blood samples (IRMA method) and IR by euglycemic insulin clamp method before TIPS, 1 day, 1 week and 1 month after TIPS. We used Wilcoxon test for statistical analysis.

Results: The insulin levels increased 1 hour after TIPS (121 vs 265 pmol/L) ($p=0.002$) and this level was stable to 1 month after TIPS. There were no differences between group of patients with and without DM. There were no changes in C-peptide level. The average metabolic clearance of glucose (M) was 1.7 mg/kg/min in diabetics and 3.7 mg/kg/min in patients without DM ($p=0.03$). There were increase of IR 1 day after TIPS ($p=0.02$) and decrease of IR 1 week after TIPS ($p=0.03$). We did not find any significant changes in M 1 month after TIPS. The results in diabetic patients were the same as in nondiabetic.

Conclusions: TIPS augments hyperinsulinemia in patients with LC by decreasing IHC. TIPS does not aggravate the insulin resistance.

Supported by IGA MZ CR NA 4558-3 and CEZ:J 13/98:111500003

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A 3-DAY INSULIN-INDUCED NORMOGLYCEMIA IMPROVES CARBOHYDRATE OXIDATION IN TYPE 2 DIABETIC SUBJECTS.

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Background and aims: 2 months of a better glycemic control improve carbohydrate oxidation in type 2 diabetes, however this benefit is uncertain for a shorter duration: hyperglycemia promotes glucose oxidation, and post-challenge glucose oxidation is reduced after a 3-day fast in such patients. We tested the effect of 3 days of normoglycemia induced by insulin infusion. **Materials and methods:** Ten type 2 diabetic subjects (age: 56 ± 3 yrs; BMI: 30.0 ± 1.1 ; HbA1c: 10.1 ± 0.5) were studied twice, before and after normal glucose levels were maintained by a 72 hours intravenous insulin infusion. Indirect calorimetry was performed 1 hour before (basal) and during the 3 hours after (post-prandial) the ingestion of a standard meal (carbohydrates: 72g, fat: 21g, protein: 32g), at 12 a.m.. Carbohydrate storage was calculated as: ingested carbohydrate - (postprandial glycosuria + suprabasal post-prandial carbohydrate oxidation). Comparisons were performed by one-way ANOVA for repeated measurements. **Results:** After normoglycemia, glucose and triglyceride levels were decreased (Basal glucose: $13.8 \pm 1.1 \text{ mmol/L}$ to 8.8 ± 0.5 ; post-prandial 14.9 ± 0.9 to 11.0 ± 0.5 ; Basal triglycerides $2.2 \pm 0.1 \text{ mmol/L}$ to 1.6 ± 0.2 ; post-prandial 2.7 ± 0.2 to 1.9 ± 0.2 ; all $p < 0.01$). C-peptides were unchanged. Glycosuria (before: 0.30 mg/kg/min) was abolished after normoglycemia. Basal carbohydrate, lipid, protein oxidation and energy production rates were unchanged. Post-prandial carbohydrate oxidation was increased after normoglycemia (before: $1.33 \pm 0.38 \text{ mg/kg/min}$, after: 1.77 ± 0.42 ; $p < 0.05$). Lipid oxidation and plasma free fatty acids tended to be more suppressed by the meal after normoglycemia (NS). Carbohydrate storage (before: 67.5 ± 4.6 g, after: 65.7 ± 3.6 g; NS) and diet-induced thermogenesis did not change after normoglycemia. **Conclusions:** Short-term insulin-induced normoglycemia improves the post-prandial oxidation of carbohydrates, but not their storage. Differing effects on lipid metabolism may explain why fasting and insulin improve carbohydrate metabolism via opposite mechanisms.

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NICOTINE INFUSION ACUTELY IMPAIRS INSULIN SENSITIVITY IN TYPE 2 DIABETIC PATIENTS BUT NOT IN HEALTHY SUBJECTS

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Background and aims: The aim of this study was to examine if an acute nicotine infusion alters insulin sensitivity to a similar degree in Type 2 diabetic patients as in healthy control subjects.

Materials and Methods: This is a double-blind, cross-over, placebo-controlled, randomised experimental study. Nicotine 0.3 µg/kg x min or NaCl was infused (two hours) during a euglycemic hyperinsulinemic clamp (four hours) to assess insulin sensitivity. Six male and female Type 2 diabetic patients (DM2; age 54±10 (mean±SD) years, BMI 25.6±2.9 kg/m²) treated with diet or one oral hypoglycemic agent and six age- and BMI-matched control subjects (Ctr). Nicotine and FFA levels, pulse rate and blood pressure were also measured.

Results: The infusions produced similar nicotine levels in both groups. In the absence of nicotine, DM2 were more insulin resistant than Ctr (6.7±0.4 vs 10.9±0.3 mg/kg LBM per min, respectively, p<0.0001). This insulin resistance was further aggravated by the nicotine infusion in DM2 but not in Ctr (4.6±0.3 vs 10.9±0.3 mg/kg LBM per min, p<0.0001). Only minor differences were seen in FFA levels, pulse rates and blood pressure.

Conclusions: At this low infusion rate, nicotine aggravated the insulin resistance in DM2 but not in Ctr. This finding may be due to the (dysmetabolic) diabetic state per se or to an increased sensitivity to environmental factors associated with a genetic predisposition for Type 2 diabetes. These results show that diabetic subjects are particularly susceptible to the detrimental effects of nicotine.

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The effect of ramipril on glucose tolerance and insulin sensitivity in animal model(OLETF/LETO rats)

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Background and Aims: Angiotensin-converting enzyme inhibitors(ACEI) are known that reduce the rates of death, myocardial infarction, stroke, heart failure as well as the risk of complications related to diabetes and of diabetes itself. But any proof is shown that ACEI improve glucose tolerance or insulin sensitivity or reduce the incidence of onset of diabetes.

Materials and Methods: To study about this issue, 4 groups of rats were studied in parallel for 6 months. OLETF rats were randomized to treat with water solution of ramipril(5mg/Kg) daily(n=10) and with saline(n=10, control group). LETO rats were also randomized as OLETF rats. We have assessed blood level of glucose, body weight, systolic and diastolic blood pressure every 1 month. After sacrifice, pancreas was obtained for the evaluation of the difference of their islet architectures.

Results: At 6 months, 24hrs urine protein level was measured and insulin tolerance test and oral glucose tolerance test were done for all experimental groups. Ramipril treatment for a period of 6 months significantly improved the mean value of AUCg(571.9±72.2 vs 629.1±79.0mg/dl), systolic blood pressure(108.2±10.6 vs 153.5±9.0mmHg), diastolic blood pressure(87.0±9.0 vs 121.9±8.4mmHg) and KITT(9.87±1.2 vs 8.97±1.9) in OLETF rats(p<0.05). Such a similar beneficial effects were also observed in the case of LETO rats. In morphologic study, prominent destructive changes of islet including islet fibrosis were observed in control groups.

Conclusions: The observed beneficial effects of ramipril on insulin sensitivity in experimental rats suggest that ramipril, long acting angiotensin-converting enzyme inhibitor, may contribute to preserve the beta cell mass and prevent the onset of diabetes itself in addition to development of diabetic complications.

PS 45

Incretin Hormones

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Daily rhythms in plasma glucagon are mediated by the biological clock and independent of food intake.

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Background and Aims: Previously we showed the existence of an endogenous rhythm in rat plasma glucose, independent of the timing food intake. The glucose rhythm was shown to be generated by the hypothalamic biological clock, located in the suprachiasmatic nucleus (SCN). At present it is not known, however, how the SCN effectuates its control. Glucagon is a pancreatic hormone strongly related to plasma glucose concentrations. Our goal was to determine whether plasma glucagon levels display a daily rhythm and, if yes, whether this rhythm is controlled by the SCN.

Materials and Methods: Male Wistar rats, equipped with permanent silicon heart catheters, were fed ad libitum or fasted for 32 hours. Plasma glucagon concentrations were determined in blood samples taken every hour for a duration of 24 hours.

Results: In ad libitum fed rats, a clear difference between glucagon concentrations in the light (mean 78 ± 5 pg/ml) and dark (mean 90 ± 5 pg/ml) period was shown. Food restriction starting at the beginning of the dark period caused a gradual decrease in glucagon levels from 81 ± 8 pg/ml in the middle of the dark period (6 hour fast), to a nadir of 56 ± 7 pg/ml in the middle of the light period (19 hour fast). Although food was still absent, glucagon concentrations gradually increased thereafter to a peak of 97 ± 11 pg/ml at the start of the following dark period. In the light period, glucagon concentrations in fasted animals were significantly lower than those in fed animals. There was no significant difference between glucagon levels of fed and fasted animals in the dark period.

Conclusions: Plasma glucagon concentrations show a daily rhythm in ad libitum fed, but also in fasted rats. The latter finding indicates the presence of an endogenous glucagon rhythm, independent of food intake. We suggest this rhythm is generated by the SCN. This hypothesis will be tested in further experiments. This work was supported by the Dutch Diabetes Foundation.

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Characterization of a miniglucagon-generating activity in the GH4C1 cell line

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Background and Aims: Miniglucagon, the C-terminal (19-29) fragment of glucagon, processed from the mother-hormone at the Arg17-Arg18 site and present in glucagon-secreting A-cells, displays original biological activities. In particular, it inhibits at very low doses (sub-picomolar) secretagogue-induced insulin secretion. A miniglucagon-generating endopeptidase (MGE) activity was shown to be present in various tissues, including liver and heart and partially characterized. However, the precise nature of MGE is still unknown.

Materials and Methods: GH4C1 cells were incubated in serum-free DMEM containing 4.5 g/l glucose for 2-hour periods. The conditioned media were pooled and, after concentration by centrifugal filtration, incubated with glucagon for various periods of time and conditions. The miniglucagon production was measured using a specific radio-immunoassay.

Results: A MGE activity is present in the GH4C1 pituitary cell line and secreted into the medium. The activity, showing an optimal pH of 7.5, displays the features of a zinc-metalloprotease: inhibition by metal chelators such as 1,10 phenanthroline (inhibition >90% at 0.1 mM) or EDTA (inhibition >60% at 1 mM) and by phosphoramidon (inhibition >60% at 50 µM), restoration of the activity by addition of zinc, while addition of large amounts of zinc in the absence of chelator inhibits the enzymatic activity (>50% at 10 mM), as observed for several zinc-metalloproteases. A weak inhibitory effect was observed with serine-proteases inhibitors such as PMSF (15% at 0.1 mM), while a modest, yet significant effect of leupeptin was observed (30% at 0.1 mM), suggesting the presence of a free thiol residue close to the active site. A prominent feature of this MGE activity is its ability to be inhibited by insulin (36% inhibition at 100 nM, >60% at 400 nM). Finally, the miniglucagon-producing activity is very sensitive to aminopeptidase A and B inhibitors such as amastatin (inhibition >60% at 1 µM) and bestatin (inhibition >60% at 30 µM).

Conclusions: Altogether, our data suggest that MGE is a complex of two metalloproteases: a zinc endopeptidase that cleaves between the two Arg residues, and an aminopeptidase that eliminates the N-terminal Arg residue of the C-terminal fragment. Such a two-step peptide processing was previously described for the NRD convertase + Aminopeptidase B complex from testis, suggesting that MGE uses the same type of processing mechanism.

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GLP-1 INDUCES PI3K ACTIVATION IN RAT ADIPOCYTES

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Background and Aims: GLP-1 has shown to be lipogenic, and also -at higher concentrations- lipolytic, in rat and human isolated adipocytes; at high doses, it provokes an increase in cAMP content, whereas no change is detected at lipogenic and non-lipolytic concentrations; in 3T3-L1 cells, a decrease in cAMP levels was documented with GLP-1, exendin-4 (Ex4), and exendin 9-39 (Ex9) -a known antagonist of pancreatic GLP-1 receptor-, which led to the concept that in these cells the GLP-1 receptor is different from the pancreatic one. In this work, the role of PI3K activation in the signal-transduction pathway leading to the effects of GLP-1 and exendins, in rat adipocytes, was investigated. **Materials and Methods:** Adipocytes were isolated by enzymatic digestion from normal Wistar rats. Lipogenesis was measured as ^{14}C -Na acetate incorporation in 2 hours, at 37°C, in the absence (control) and presence of 10^{-13} - 10^{-9} M Ex4 or Ex9, or 10^{-10} - 10^{-9} M GLP-1 or insulin, and without and with 10^{-6} M wortmannin (PI3K inhibitor). Glycerol release was measured in the absence and presence of 10^{-9} M GLP-1, without and with wortmannin, during 60 min at 37°C. PI3K activity was measured as PIP3 formation in cells incubated during 3 min in the absence and presence of 10^{-9} M GLP-1, Ex4 or Ex9. **Results:** Ex4 and Ex9, both significantly stimulated lipogenesis from already 10^{-13} M (Ex4: $124 \pm 7\%$ of control, $p < 0.005$; Ex9: 125 ± 4 , $p < 0.01$; both $n=5$), reaching their apparent maximal value at 10^{-10} M (Ex4: 143 ± 10 , $n=6$, $p < 0.02$; Ex9: 141 ± 6 , $n=5$, $p < 0.01$), and being the stimulus at 10^{-10} M similar to that exerted by GLP-1 (137 ± 7 , $n=4$, $p < 0.01$) or by insulin (124 ± 5 , $n=5$, $p < 0.02$). The presence of wortmannin abolished the lipogenic action of GLP-1 as well as that of insulin or either exendin. The effect of 10^{-10} M GLP-1 on glycerol release ($223 \pm 15\%$ of control, $n=5$, $p < 0.001$) was reduced in the presence of wortmannin (142 ± 14 , $n=5$, $p < 0.05$, and $p < 0.05$ vs GLP-1). Also, 10^{-10} M GLP-1, Ex4 and Ex9, as insulin, induced a significant ($p < 0.01$, or lower) activation of PI3K. **Conclusions:** In rat adipocytes, the lipogenic effect of GLP-1, as that of insulin, requires the activation of PI3K, whereas its lipolytic effect is only partially reduced in the presence of wortmannin. Ex4 and Ex9, both induce lipogenesis, and for their lipogenic action the activation of PI3K is also required.

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ETHANOL EXAGGERATES POSTPRANDIAL TRIGLYCERIDE LEVELS AND DEALYS GLP-1 RESPONSES IN TYPE-2 DIABETIC PATIENTS

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Background and Aims: Alcohol is known to increase triglyceride levels in both healthy and diabetic subjects. Increased fasting triglyceride concentrations are found to be an independent risk marker of cardiovascular disease. Our aim was to study the impact of alcohol on postprandial lipemia in type-2 diabetic subjects by comparing the postprandial responses of triglycerides, NEFA, glucose, insulin and the incretins, glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP), to three fat rich test meals. **Material and Methods:** In a randomised cross-over trial 11 subjects with type-2 diabetes (62.8 ± 7.1 years; BMI 28.9 ± 3 kg/m 2 ; HbA1c $6.4 \pm 0.8\%$) were studied on 3 separate days, where the following meals were ingested in a random order: 100 g butter (control), 100 g butter+40 g alcohol and 50 g carbohydrate (alco), 100 g butter+120 g carbohydrate (CHO). The alco and CHO were isoenergetic. The postprandial test period was 8 h. Repeated Measurements of ANOVA was used for statistical calculations. **Results:** Alco induced significantly higher triglyceride responses at 6 h and 8 h compared with the two other test meals which did not cause different responses. Mean values \pm SD at T=360 min: (alco: 4.0 ± 2.3 ; control: 2.8 ± 1.7 ; CHO: 3.2 ± 1.9) mmol/L. Mean values \pm SD at T=480 min: (alco: 3.8 ± 2.3 ; control: 2.2 ± 1.2 ; CHO: 2.4 ± 1.8) mmol/L. Incremental area under the postprandial 8 h curve (IAUC-8h) for the serum glucose curve was increased by 3.8 times to CHO and by 2.0 times to alco compared to the control ($P < 0.05$). IAUC-8h for insulin was 3 times higher to CHO compared to control ($P < 0.05$); no significant difference between control and alco. IAUC-8h for GIP to CHO was 1.7 times higher compared to control and 1.5 times greater than alco. No statistical significant difference was seen between control and CHO. GLP-1 was significantly suppressed by alco at T=60 min compared with control and CHO, mean values \pm SD: (Alco: 16 ± 6 ; Control: 27 ± 12 ; CHO: 30 ± 9) pmol/L. At T=480 min the GLP-1 response was significantly increased compared with control and CHO (alco: 33 ± 14 ; control: 20 ± 4 ; CHO: 18 ± 10) pmol/L. **Conclusion:** Supplementation of a fat rich meal with alcohol in type-2 diabetic subjects increased the triglyceride responses and suppressed the GLP-1 responses without affecting insulin levels. Whether this reflects a direct alcohol-induced suppression of GLP-1 which in turn cause an impairment of triglyceride clearance in type-2 diabetic patients remains to be elucidated.

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THE CEPHALIC INSULIN RELEASE IS CHOLINERGIC AND NON-CHOLINERGIC AND OF PHYSIOLOGICAL IMPORTANCE IN HUMANS

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Background. The preabsorptive insulin response to meal intake has in animals been demonstrated to be cholinergically mediated. However, its nature as well as its physiological relevance for the glucose homeostasis have not been established in humans. We therefore studied the mechanisms and physiological relevance of the cephalic insulin response to meal ingestion in twelve healthy volunteers.

Subjects and methods. Twelve healthy women (age 63 ± 0.4 years, BMI 27.7 ± 1.7 kg/m 2) were given either the ganglionic antagonist, trimetaphane, which impairs neurotransmission across parasympathetic and sympathetic autonomic ganglia, or atropine or saline during the first 15 min after ingestion of a standard meal (350 kcal). Frequent samples were taken for analysis of glucose, insulin and the gastrointestinal hormones gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). **Results.** During saline infusion, insulin increased during the first 10 min after meal ingestion, whereas the first increase in glucose was evident at min 15. The preabsorptive 10 min insulin response was reduced by $73 \pm 11\%$ by trimetaphane ($P = 0.009$), which was accompanied by impaired reduction of glucose levels from min 25 to 60 after meal ingestion (Δ glucose with saline -1.27 ± 0.5 mmol/l versus $+0.1 \pm 0.4$ mmol/l with trimetaphane, $P = 0.008$). This 25 to 60 min reduction in glucose levels correlated significantly to the 10 min insulin response ($r = 0.65$, $P = 0.024$). The 10 min insulin response to meal ingestion was also reduced by atropine, but only by $20 \pm 9\%$ ($P = 0.045$), which was lower than by trimetaphane ($P = 0.004$). The preabsorptive insulin response was not accompanied by increases in GIP or GLP-1.

Conclusion: 1) The early preabsorptive insulin response to meal ingestion in humans is largely due to autonomic activation mediated by both non-cholinergic and cholinergic mechanisms, 2) this cephalic insulin response is required for a normal glucose tolerance, and 3) GIP and GLP-1 do not contribute to the preabsorptive cephalic phase insulin response.

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DIPEPTIDYL PEPTIDASE IV INHIBITION PROMOTES DIFFERENTIATION OF NEW BETA CELLS IN 60% PANCREATOTOMISED RATS

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Background and aims: Inhibition of dipeptidyl peptidase IV (DPP-IV) is known to protect the endogenous intact biologically active form of glucagon-like peptide 1 (GLP-1) from degradation, and to have an ameliorative effect on glucose tolerance in rats. Since GLP-1 is believed to have an effect on beta-cell mass, we have investigated the effect of valine pyrrolidide (VP) in 60% pancreatectomised rats. **Materials and Methods:** Male Sprague-Dawley rats (100 g) were subjected to 60% pancreatectomy and allowed to recuperate for 4 days. Animals were divided into two groups ($n=6$) with matched oral glucose tolerance (OGTT, 2g/kg) on day 4 and were then treated with VP 20 mg/kg or vehicle (water) p.o. twice daily for 4 days. On day 8 a second OGTT was performed, and, on day 9 a blood sample was drawn and remnant and regenerated pancreas was removed and immunostained for insulin. **Results:** The area under the glucose curve during OGTT (0-120 min) is reduced significantly in the VP treated animals to $51 \pm 32\%$ of pre-dose OGTT ($p < 0.05$). The vehicle treated group showed an unchanged area, $100 \pm 35\%$. The single blood sample drawn just before sacrifice showed elevated plasma GLP-1 concentration level in the VP treated group (25 ± 6 pM vs. 15 ± 2 pM, $p < 0.05$) and slightly elevated insulin concentration level too (191 ± 81 pM vs 131 ± 17 pM, ns.). Histological evaluation revealed that in 5 out of 6 VP treated animals, insulin expression in beta-cells was greater in islets located in the regenerating tissue. In the vehicle group insulin expression was detected in only 1 out of 6 animals. **Conclusion:** The results indicate that the DPP-IV inhibitor VP accelerates the differentiation process in the regenerating tissue, leading to increased formation of new beta-cells. This result is consistent with the significant decrease of the glucose excursion observed with VP treatment in this model.

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Cultured pancreatic ductal cells undergo cell cycle re-distribution and beta-cell-like differentiation in response to GLP-1.

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Background and Aims: The intestinal hormone GLP-1 has been shown to promote an increase in pancreatic beta cell mass via proliferation of islet cells and differentiation of non-insulin secreting cells. In this study we characterize some of the events that lead to the differentiation of pancreatic ductal cells in response to treatment with human GLP-1.

Materials and Methods: Rat pancreatic ductal cells (ARIP) were cultured in the presence of GLP-1 and analyzed for cell cycle distribution, expression of growth arrest molecules p21 and p27, and transcription of beta-cell-specific genes.

Results: Exposure of ARIP cells to 10 nM of GLP-1 induced a cell cycle re-distribution leading to a significant growth arrest of cell in culture. Comparison of serum-starved cells with GLP-1-treated cells (for 12 h) showed a significant decrease of cells in S-phase. This was associated with a symmetrical increase of the percentage of GLP-1-treated cells in G0-G1-phase of the cell cycle. Western-blot analysis for the growth arrest signaling proteins p21 and p27, demonstrated that GLP-1 induced an up-regulation of both factors. A significant increase of p21 level was observed within the first 12 h from the beginning of GLP-1-treatment, while p27 reached its highest expression level at 24h. As cells slow down their proliferation-rate, GLP-1 induced a time-dependent expression of various beta-cell-specific mRNAs. The glucose transporter GLUT-2 was always the 1st of those factors to be expressed (24 h of GLP-1 treatment), followed by insulin (48 h) and finally by the glucose phosphorylating enzyme glucokinase (56 h).

Conclusions: The present study elucidates some of the biological steps that lead a ductal cell to acquire a beta-cell-like phenotype.

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EVIDENCE FOR EARLY IMPAIRMENT OF INCRETIN-INDUCED INSULIN SECRETION IN TYPE 2 DIABETES.

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Background and Aims: To investigate a possible role of an entero-insular axis involvement in the pathogenesis of type 2 diabetes, plasma Glucagon-Like Peptide 1 (GLP-1) 7-36 amide response to nutrient ingestion was evaluated in type 2 diabetics affected by different degrees of beta-cell dysfunction. **Materials and Methods:** 14 patients on oral hypoglycaemic treatment (Group A: HbA1C=8.1±1.8%) and 11 age-matched diabetics on diet only (Group B: HbA1C=6.4±0.9) participated in the study. 10 healthy volunteers were studied as controls. In postabsorptive state a mixed meal (700 KCal) was administered to all subjects and blood samples were regularly collected until 180' for plasma glucose, insulin, glucagon and GLP-1 determination. **Results:** In the control group the test meal induced a significant increase in plasma GLP-1 at 30' and 60' (p<0.01), returning then the peptide values toward basal levels. B-cell function estimation by HOMA score confirmed a more advanced B-cell involvement in Group A with respect to Group B (p<0.01), insulin resistance degree resulting, by contrast, similar between the groups (HOMA-R). In Group A first phase postprandial insulin secretion (0-60') was, as expected, significantly reduced with respect to healthy subjects (AUC: p<0.001). In the same patients mean fasting GLP-1 values was similar to controls, but the meal failed to increase plasma peptide levels, which even tended to decrease during the test (p<0.01). In group B food-mediated early insulin secretion was increased with respect to group A (AUC: p<0.001), even though significantly reduced when compared to controls (p<0.01). Similarly to group A, no GLP-1 response to food ingestion occurred in Group B patients, in spite of maintained basal peptide secretion. Whereas in the control group the test meal did not significantly modify plasma glucagon levels, in both groups of diabetic patients glucagon concentrations increased at 30' and 60' (p<0.01). **Conclusions:** 1) the incretin effect, in terms of GLP-1 secretion, results to be seriously impaired even in condition of mild diabetes; 2) the early entero-insular axis dysfunction could play a role in the progressive B-cell deterioration towards overt type 2 diabetes.

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INHIBITORY EFFECT OF GHRELIN ON INSULIN SECRETION IN THE RAT PANCREAS.

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Background and Aims: Ghrelin is a novel 28 amino acid peptide identified in both rat and human stomach which acts as an endogenous ligand for the growth-hormone secretagogue receptor (GHS-R) and stimulates growth hormone (GH) release. GHS-R is expressed in a number of tissues including the pancreas. Ghrelin has been found in peripheral blood. Interestingly, in the rat, plasma ghrelin levels are increased by fasting. In the present study we have investigated the effect of ghrelin on insulin and glucagon secretion in the isolated perfused rat pancreas. **Materials and Methods:** Perfusate consisted of Krebs-Henseleit buffer supplemented with dextran (4%), albumin (0.5%) and glucose (5.5 mmol/l). Hormones were measured by RIA. **Results:** Infusion of ghrelin at 2 nmol/l significantly reduced the late phase (from 5 to 15 min) of the insulin response to increasing perfusate glucose level from 5.5 to 9 mmol/l (incremental area: 34±6 ng/10 min, Mean±SEM, vs. 80±16 ng/10 min in control experiments; p<0.05). At 10 nmol/l ghrelin markedly inhibited both phases of the insulin response to glucose [incremental areas: First phase (0-5 min): 11±2 ng/5 min vs. 27±4 ng/5 min, in control experiments; p<0.05. Second phase (5-15 min): 43±14 ng/10 min vs. 79±10 ng/10 min in controls; p<0.05]. Ghrelin also reduced the late phase (from 5 to 15 min) of the insulin response to 10 mmol/l arginine (incremental area: 78±16 ng/10 min vs. 176±33 ng/10 min in control experiments; p<0.05). Ghrelin did not significantly affect the glucagon response to arginine. **Conclusion:** Our findings are consistent with a direct inhibitory effect of ghrelin on the B-cell. At present, conjecture about the implication of this peptide in the regulation of insulin secretion would be highly speculative. However, when considering ghrelin as a pharmacological agent – by virtue of its GH releasing activity, its diabetogenic (insulinostatic) effect should be taken into account.

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Effect of xenin-8 on pancreatic hormone secretion in the rat pancreas.

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Background and Aims: Xenin is a 25 amino acid peptide of the neurotensin/xenopsin family identified in gastric mucosa and brain in various mammals including man. In healthy subjects, plasma xenin immunoreactivity increases after meals. Moreover, in dogs, i.v. xenin infusion raises plasma levels on insulin and glucagon and stimulates exocrine pancreas secretion. The latter effect has also been demonstrated for xenin-8, the C-terminal octapeptide of xenin. We have investigated the direct effect of xenin-8 on insulin, glucagon and somatostatin secretion in the perfused rat pancreas.

Materials and Methods: Pancreases were isolated from fed normal Wistar rats. Perfusate consisted of Krebs-Henseleit buffer supplemented with dextran (4%), albumin (0.5%) and glucose (5.5 mmol/l). Xenin-8 (Peninsula Labs.) was tested at 100 nmol/l. Hormones were measured by RIA.

Results: Xenin-8 infusion induced a prompt, short-lived increase in insulin secretion at 5.5 mmol/l glucose (F10,50=2.51; p<0.05) and potentiated the insulin response to increasing perfusate glucose concentration from 5.5 to 9 mmol/l (incremental area: 56±3 ng/15 min, Mean±SEM, vs. 32±5 ng/15 min in control experiments; p<0.01). The inhibition of glucagon release induced by such glucose increase (F15,45=3.01; p<0.05) was not observed when xenin-8 was simultaneously infused (F15,75=0.77; n.s.). Xenin-8 failed to significantly modify somatostatin release (F10,40=0.2; n.s.).

Conclusions: In the rat pancreas, the C-terminal octapeptide of xenin stimulates insulin release, counteracts the glucagonostatic effect of increasing glucose level and does not affect somatostatin output. Our findings support the concept that the reported 'in vivo' effects of xenin on insulin and glucagon plasma levels represent a direct influence of this peptide on B- and A-cells.

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Lowered blood glucose, hyperglucagonemia and pancreatic alpha-cell hyperplasia in glucagon receptor deficient mice.

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Background and Aims: Glucagon, the counter regulatory hormone to insulin, is secreted from the pancreatic alpha-cells in response to low blood glucose. The main function of glucagon is to stimulate hepatic glucose production. However, glucagon receptors (GR) are found on a number of extra-hepatic tissues suggesting other regulatory roles. To examine in detail the role of glucagon in glucose homeostasis we generated mice homozygous for a null deletion of the glucagon receptor (GR^{-/-}).

Materials and Methods: Mice homozygous for a null deletion of the GR were generated using standard gene targeting technology. In all studies described here, GR^{-/-}, GR^{+/-} and littermate wild type (WT) control mice back-crossed (F3-F6) into the C57BL6 background were used. All studies were carried out following the NIH Principles of laboratory animal care.

Results: GR^{-/-} mice appeared normal and had similar growth curves to both WT control and GR^{+/-} mice. Northern analysis of liver RNA, [¹²⁵I]-glucagon binding studies of liver membranes, and conscious glucose challenge experiments all indicated that GR^{-/-} mice lacked functional GR. Mean daily blood glucose levels in GR^{-/-} mice were 68% of that seen in WT or GR^{+/-} mice, and fasting blood glucose levels were 4.1 ± 0.2 mM for GR^{-/-} compared to 6.9 ± 0.3 mM in age, sex matched WT control mice. Serum glucagon was elevated to supra-physiological levels in GR^{-/-} mice: GR^{-/-}, 23500 ± 590 pg/ml vs. WT, 60 ± 6 pg/ml. Immunocytochemical examination revealed vast alpha-cell hyperplasia in the pancreatic islets of GR^{-/-} compared to either WT controls or GR^{+/-} mice, with the alpha-cell mantle of most islets accounting for approximately 60-80% of the total islet mass.

Conclusions: Preliminary characterization of GR^{-/-} mice indicates that while these mice are viable they display lowered blood glucose throughout the day. In addition serum glucagon levels are extremely elevated due to substantial hyperplasia of alpha-cells within the pancreatic islets. Studies are underway to more fully characterize alterations in glucose homeostasis in GR^{-/-} mice and to determine the molecular mechanism responsible for the alpha-cell hyperplasia.

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CHARACTERIZATION OF GLUCAGON RECEPTOR KNOCKOUT MICE.

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Background and Aims: The action of glucagon in the liver is mediated by G-coupled receptors coupled both to adenylate cyclase and to the hydrolysis of inositol phospholipids. In order to examine the role of glucagon in glucose homeostasis we have generated mice in which the glucagon receptor was inactivated (GR^{-/-} mice).

Materials and Methods: The targeting vector was constructed by cloning 8.4 Kb of 5' homology and 2.9 Kb of 3' homology from a DBA/1LacJ murine genomic phage library into a pJNS2 (PGK-NEO/PGK-TK) backbone vector. The glucagon receptor knockout vector replaced ~1.8 Kb of genomic locus, corresponding to amino acids 56-303. Deletion of the glucagon receptor was confirmed by RT-PCR using liver tissue and by measurement of [¹²⁵I]-glucagon binding to liver membranes.

Results: GR^{-/-} mice were of similar body weight to age-matched wild-type (WT) animals. Fasting blood glucose levels were somewhat elevated in GR^{-/-} mice relative to WT (83 ± 5 mg/dl in WT, 136 ± 7 mg/dl* in GR^{-/-}) as were fasting plasma insulin levels (0.42 ± 0.07 ng/ml in WT, 0.66 ± 0.18 ng/ml* in GR^{-/-}). All data are means \pm SEM for 4-14 male animals, * indicates $p < 0.05$ vs. WT by t-test. The corresponding values in fed mice were blood glucose 104 ± 1 mg/dl in WT, 119 ± 3 mg/dl* in GR^{-/-}; plasma insulin 2.85 ± 0.94 ng/ml in WT, 0.86 ± 0.15 ng/ml* in GR^{-/-}. There was no significant effect on fasting plasma cholesterol (156 ± 5 mg/dl in WT, 140 ± 9 mg/dl in GR^{-/-}) or triglyceride levels (197 ± 11 mg/dl in WT, 159 ± 26 mg/dl in GR^{-/-}) associated with deletion of the glucagon receptor. Glucose tolerance, as assessed by an oral glucose tolerance test, was normal. Plasma glucagon levels were strikingly elevated (160 ± 21 ng/ml in WT, 4222 ± 487 ng/ml* in GR^{-/-} when fasting, and 62 ± 9 ng/ml in WT, 1891 ± 324 ng/ml* in GR^{-/-} when fed).

Conclusions: Mice in which glucagon receptors are not expressed maintain near-normal glycemia and normal lipidemia, in the presence of circulating glucagon concentrations that are elevated by 2 orders of magnitude.

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GIP Analogues

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A fast GIP bolus injection test to characterize insulin secretory defects in first degree relatives of type 2 diabetic patients

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Background and Aims: Since the responsiveness of insulin secretion to exogenous Gastric inhibitory Polypeptide (GIP) is reduced in normal glucose-tolerant first-degree relatives under hyperglycaemic clamp conditions, the question arose whether simpler tests might be applicable.

Patients and Methods: 25 first-degree relatives of type 2-diabetic patients (age 44 ± 11 y., BMI 25.7 ± 4.4 kg/m²) and 14 healthy control subjects (negative family history, 45 ± 12 y., BMI 25.7 ± 2.8 kg/m²) were examined with an oral glucose load (75 g) and an intravenous bolus injection of synthetic human GIP, 20 pmol/kg body weight administered on different occasions in the fasting state. Blood was drawn over 35 min. for plasma glucose (glucose oxidase), insulin, C-peptide (specific immunoassays). Statistics: RM-ANOVA.

Results: Insulin secretion (insulin, C-peptide plasma levels) was stimulated significantly with exogenous GIP ($p < 0.0001$). The insulin response was reduced in first degree relatives compared to normal subjects ($p < 0.005$) and a significant proportion of first degree relatives fell below the 95% confidence interval of healthy subjects. Similar results were obtained with C-peptide. However the difference was not significant while the pattern was identical.

Conclusion: In line with a lower insulin secretory response to exogenous GIP under hyperglycaemic clamp conditions, our quick bolus injection test identified subjects with a reduced insulin secretory response towards GIP in a similar way. Such a test may be of value to detect early insulin secretion defects in a population at risk for developing type 2 diabetes.

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DPP IV RESISTANT N-TERMINALLY MODIFIED GIP ANALOGUES WITH ENHANCED INSULINOTROPIC ACTIVITY

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Aims: To examine the plasma stability and biological activity *in vitro* of two novel N-terminally modified analogues of GIP, acetylated GIP (Ac-GIP) and pyroglutamyl GIP (pGlu-GIP). **Methods:** GIP, Ac-GIP and pGlu-GIP were synthesised by Fmoc solid-phase peptide synthesis, purified by HPLC and identified by electrospray ionization mass spectrometry. Degradation studies were carried out by incubating each peptide (n=3) with either DPP IV or human plasma. Insulin releasing ability (mean \pm SEM, n=8) was assessed by acute 20 min incubations with clonal pancreatic BRIN-BD11 cells. Cyclic AMP production was assessed (n=6) in Chinese hamster lung fibroblast (CHL) cells transfected with human GIP receptors. **Results:** GIP was rapidly degraded by DPP IV and plasma, the half-lives being 2.3 and 6.2 h, respectively. In contrast, no degradation product GIP(3-42) was observed following exposure of Ac-GIP or pGlu-GIP to DPP IV or plasma even after 24 h. Native GIP dose-dependently stimulated insulin secretion by 1.2 to 1.8-fold over the concentration range 10^{-13} to 10^{-8} mol/l at 5.6 mmol/l glucose. Both Ac-GIP and pGlu-GIP were more potent at stimulating insulin release between 10^{-11} to 10^{-8} mmol/l when compared to the native GIP ($P < 0.001$), with 1.4-fold and 1.3-fold increases observed for Ac-GIP and pGlu-GIP at 10^{-8} mol/l, respectively. Both Ac-GIP and pGlu-GIP were extremely potent ($P < 0.001$) at stimulating cAMP production with EC₅₀ values of 1.9 and 2.7 nmol/l, respectively, almost a 10-fold increase when compared to native GIP (18.2 nmol/l). The maximal cAMP production when compared to GIP for both Ac-GIP and pGlu-GIP were $165.7 \pm 1.3\%$ and $183.9 \pm 5.7\%$, respectively. **Conclusions:** N-terminal modifications of GIP confer not only resistance to plasma degradation but significantly increase biological activity *in vitro*, raising the possibility of use in therapy of type 2 diabetes.

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GIP ANALOGUES SUBSTITUTED AT Ala² EXHIBIT IMPROVED PLASMA STABILITY AND INSULIN-RELEASING ACTIVITY

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Aims: To determine the plasma stability, insulinotropic activity and cyclic AMP production of two novel analogues of GIP substituted at the penultimate Ala² by Gly or Ser. **Methods:** GIP, (Ser²)GIP and (Gly²)GIP were synthesized using standard Fmoc solid-phase peptide synthesis, purified by reversed-phase HPLC and characterised using electrospray ionization mass spectrometry. Peptide degradation was determined by incubation *in vitro* with both dipeptidylpeptidase IV (DPP IV) and human plasma. Insulin release (mean \pm SEM, n=8) was measured from clonal pancreatic BRIN-BD11 cells following acute 20 min incubations. Intracellular cAMP production was measured (n=6) using Chinese hamster lung fibroblast (CHL) cells stably transfected with human GIP receptors. **Results:** Incubation with DPP IV showed the half-lives of (Ser²)GIP and (Gly²)GIP to be significantly greater than native GIP 4.8, >12 and 2.3 h, respectively. In human plasma, the half-lives were 9.8, >12 and 6.2 h, respectively. Native GIP dose-dependently stimulated insulin secretion by 1.2- to 1.8-fold over the concentration range 10⁻¹³ to 10⁻⁸ mol/l at 5.6 mmol/l glucose. Both (Gly²)GIP and (Ser²)GIP were more potent at stimulating insulin secretion when compared to the native GIP (P<0.001), with 1.2-fold and 1.5-fold increases observed at 10⁻⁸ mol/l, respectively. Upon binding to CHL cells, GIP, (Ser²)GIP and (Gly²)GIP evoked a marked stimulation of cAMP production. The calculated EC₅₀ values for these peptides were 18.2, 15.0 and 14.9 nmol/l, respectively. **Conclusions:** Substitution at the penultimate Ala² in GIP by Ser or Gly not only improves stability to plasma degradation by DPP IV but enhances biological activity *in vitro* suggesting a possible role in type 2 diabetes therapy.

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The effect of gastric inhibitory polypeptide on 3T3-L1 adipocytes.

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Background and Aims: Gastric inhibitory polypeptide (GIP) is an insulinotropic peptide released from intestinal K cells after absorption of glucose or fat. We have revealed that GIP is essential in promoting obesity when fed a high fat diet using GIP receptor deficient mice. In this study, we have examined the direct effects of GIP on adipocytes by using differentiated 3T3-L1 cells.

Materials and Methods: 3T3-L1 cells were differentiated with insulin, IBMX and dexamethasone. The cAMP content of the cells was measured after 30-min incubation with or without GIP. The uptake of 2-deoxyglucose was measured by using 2-[3H(G)]deoxyglucose. Lipoprotein lipase (LPL) activity was measured in medium after 3-h incubation with or without GIP followed by addition of heparin.

Results: GIP failed to affect the cAMP levels in undifferentiated 3T3-L1 cells. However, the cAMP production in adipocytes derived from 3T3-L1 cells was increased to 8.4-fold by the stimulation of 1 μ M GIP. In contrast to the case of GIP, glucagon-like peptide-1 (GLP-1), another incretin, had no effects on cAMP levels even in differentiated 3T3-L1 cells. GIP also significantly increased 2-deoxyglucose uptake in 3T3-L1 adipocytes both in the absence (54.8 \pm 2.2 pM/min/well at 0 M GIP vs. 66.9 \pm 5.0 at 100 nM GIP) and presence (109.3 \pm 8.2 vs. 174.7 \pm 13.6) of 1 nM insulin. Moreover, GIP at 1 nM increased an activity of the LPL on 3T3-L1 adipocytes by 57%.

Conclusions: The results in this study suggest that the stimulation by GIP could increase lipid accumulation in 3T3-L1 adipocytes through the production of cAMP. These effects of GIP on adipocytes indicate that GIP promotes obesity not only through stimulation of insulin secretion from pancreatic β -cells but also through a direct effect on adipocytes.

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ANTAGONIST ACTIONS OF NOVEL (Pro³)GIP ANALOGUE AND GIP(3-42) ON CYCLIC AMP PRODUCTION AND INSULIN SECRETION

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Aims: To determine the biological activity of a truncated and a structurally modified GIP analogue on cyclic AMP production and insulin secretion. **Methods:** GIP(1-42), (Pro³)GIP and GIP(3-42) were synthesised by Fmoc solid-phase peptide synthesis, purified by HPLC and characterised by electrospray ionization mass spectrometry. Both GIP and (Pro³)GIP were tested for stability by incubation (n=3) with pooled human plasma and dipeptidylpeptidase IV (DPP IV). Insulin secretion (mean \pm SEM) was assayed using cultured glucose-responsive BRIN-BD11 cells during acute 20 min incubations (n=8). Intracellular cAMP stimulation at the GIP receptor was measured using Chinese hamster lung fibroblast cells (n=6) transfected with the human GIP receptor. **Results:** GIP was rapidly degraded by DPP IV and human plasma with only 62 \pm 5% and 23 \pm 1% remaining intact after 4 h. (Pro³)GIP proved to be a DPP IV resistant analogue and remained intact over the 24 h incubation as determined by HPLC. In clonal BRIN-BD11 cells, GIP (10⁻¹³ to 10⁻⁸ mol/l) stimulated insulin secretion (1.2- to 1.8-fold; P<0.01) compared to control incubations (5.6 mmol/l glucose alone, n=8). Both (Pro³)GIP and GIP(3-42) were significantly less potent at stimulating insulin secretion (P<0.01) compared to control. In the presence of GIP (10⁻⁷ mol/l) both (Pro³)GIP and GIP(3-42) dose-dependently inhibited (P<0.001) insulin release compared to native GIP. At 10⁻⁸ mol/l (Pro³)GIP and GIP(3-42) exhibited 1.7- to 1.8-fold decreases in insulinotropic activity compared to native GIP. GIP was able to stimulate cAMP production with an EC₅₀ value of 18.2 nmol/l. (Pro³)GIP and GIP(3-42) only very weakly stimulated cAMP production and antagonised the effects of 10⁻⁷ mol/l native GIP. The maximal stimulatory values compared to GIP (100%) were 9 \pm 2.1% and 25 \pm 2.5%, respectively. **Conclusions:** (Pro³)GIP and GIP(3-42) acted like GIP receptor antagonists, inhibiting GIP mediated insulin release and cAMP production.

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Quantification of the incretin effect in first-degree relatives of Type 2-diabetic patients compared to healthy control subjects

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Background and Aims: A reduced responsiveness of insulin secretion to exogenous Gastric Inhibitory Polypeptide (GIP) has not only been demonstrated in Type 2-diabetic patients, but also in their normal glucose-tolerant first-degree relatives, who are characterized by a high risk to develop Type 2-diabetes later in life. It was the aim to investigate the integrity of the entero-insular axis in such subjects.

Materials and Methods: Seventeen first-degree relatives of Type 2-diabetic patients (5 male, 12 female, age 50 \pm 11 y., BMI 26.1 \pm 3.8 kg/m²) and 9 healthy control subjects (negative family history, 5 male, 4 female, p = 0.23; 45 \pm 13 y., p = 0.32; 25.7 \pm 4.3 kg/m², p = 0.74) were examined with an oral glucose load (75 g; O) and an 'isoglycaemic' intravenous glucose infusion (IV) copying the same glycaemic profile on separate occasions starting in the fasting state. Blood was drawn over 240 min for plasma glucose (glucose oxidase), insulin, C-peptide, GIP, GLP-1, and glucagon (specific immunoassays). Statistics: RM-ANOVA, regression analysis.

Results: Insulin secretion (insulin, C-peptide plasma levels) were stimulated significantly more by oral as compared to intravenous glucose in both groups. The percent contribution of the incretin effect ((O-IV)/O * 100 [%]) was similar in both groups (C-peptide: 62.5 \pm 5.1 vs. 63.6 \pm 7.1 %, p = 0.90; insulin: 74.2 \pm 3.3 vs. 74.7 \pm 5.3 %, p = 0.97). A tendency towards higher GIP and lower GLP-1 responses after oral glucose was not significant (p = 0.79 and 0.80, respectively). Integrated incremental insulin secretory responses and incretin effects correlated more significantly with GIP than with GLP-1 responses, the relations being stronger in healthy subjects than in first-degree relatives.

Conclusions: Despite a lower insulin secretory response to exogenous GIP, incretin effects are similar in first-degree relatives of Type 2-diabetic patients. This is not explained by the compensatory hypersecretion of GIP or GLP-1. GIP appears to be the more important incretin (vs. GLP-1), especially in healthy subjects.

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GLP-1 Analogues and Glucagon Antagonism

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Sustained appetite suppression and weight loss in obese rhesus monkeys treated with a long-acting GLP-1 derivative, NN2211

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Background and Aims: In rodents and pigs, the long-acting GLP-1 derivative, NN2211, has been reported to have potentially important clinical properties, including glucose lowering, and attenuation of diabetes development, as well as appetite suppression and lowering of body weight. We have examined the efficacy of NN2211 to alter ad libitum food intake levels, and to reduce body weight in adult rhesus monkeys (*Macaca mulatta*) with spontaneous naturally-occurring middle-age onset obesity.

Materials and Methods: Five non-diabetic monkeys with a mean(±SE) body weight of 15.8±1.2 kg (body fat >25%) were provided monkey chow ad libitum, with biscuits counted two times per day to assess intake in the first 1.5 hours after dosing and during the total 8 hour food intake period. The protocol included five test periods: initial vehicle (three weeks), to be followed by NN2211 (30µg/kg b.i.d.) administered by subcutaneous injection, a washout period, a second dosing level (10µg/kg b.i.d.), and a final washout period.

Results: Mean food intake during the predosing period was 722 ± 31 kcal/day (X±SE), equal to an average intake of 48.6 kcal/kg per day (range 40.1 to 53.8 kcal/kg/day). The 30µg/kg/day dose of NN2211 produced an immediate and sustained reduction in food intake to less than 10% of baseline intake (mean intake 44±9.1 kcal/day; 3 kcal/kg/day), accompanied by significant weight loss of 0.4 kg (p<0.03) over 4 days. This dose was selected based on acute studies, but produced very low intakes and was therefore halted. Food intake then recovered. NN2211 was resumed at a dose of 10µg/kg b.i.d. for 16 days (after 6 days one monkey's dose was further reduced to 5µg/kg/day due to excessive food intake reduction). NN2211 (10µg/kg b.i.d.) produced a sustained reduction in food intake to 45.7±5.2 kcal/kg/day, 38% below baseline intake (p<0.05), with weight loss during the 16 days of 0.42 kg (p<0.05). Immediately after termination of dosing food intake recovered fully (701±46 kcal per day). Body weight was regained during the month of follow up after the end of dosing. At all times the behavior of the monkeys appeared to be normal, with no signs of illness.

Conclusions: This GLP-1 derivative, NN2211, appears to have sustained activity to reduce food intake over at least 8 hours and to produce weight loss in obese rhesus monkeys, indicating strong potential for clinical efficacy in humans.

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NN2211, A LONG-ACTING GLP-1 DERIVATIVE, AMELIORATES GLYCAEMIA AND REDUCES TRIGLYCERIDE LEVELS IN PRE-DIABETIC ZDF RATS IN THE ABSENCE OF INCREASED β-CELL MASS.

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Background and Aims: GLP-1 has several effects desirable for the treatment of type 2 diabetes but has a very short half-life. NN2211, a long-acting derivative of GLP-1, is designed for once daily administration in humans. This study compared the effect of NN2211 on glycaemia, insulin and lipid levels, body weight, and β-cell mass in young ZDF rats after 2 weeks dosing.

Materials and Methods: Male pre-diabetic ZDF rats, 8 weeks old, received 200 µg/kg NN2211 or vehicle (veh) s.c. twice daily (n=10). Blood glucose and plasma insulin were measured during an OGTT after 1 and 26 doses (day 13). Basal (unfasted) levels of insulin, glucagon, fructosamine, triglyceride and total cholesterol were measured after 15 doses (day 8). Body weight was measured before the study start and at the end of the study period (day 13).

Results: NN2211 did not affect the BG response during OGTT after 1 dose, but significantly lowered AUC for glucose after 26 doses (1194±50 mmol/l*min vs 1682±117 mmol/l*min p=0.0012). Delta peak insulin was significantly increased with NN2211 (743±137 pmol/l vs 174±82 pmol/l, p=0.003). Basal insulin was significantly lower with NN2211 (742±78 pmol/l vs 1151±80 pmol/l, p=0.0017). Fructosamine levels (128.5±2.4 µmol/l vs 169.7±9.3 µmol/l, p<0.001) and triglyceride levels (3.1±0.2 mmol/l vs 9.8±1.1 mmol/l, p<0.001) were also significantly reduced with NN2211 compared with vehicle. Body weights were significantly lower in the NN2211 treated group at the end of the study period (319±5 g vs. 347±4 g, p<0.001). β-cell mass was lower in the NN2211 treated group (6.2±0.6 mg vs 9.0±0.6 mg, p<0.007) and the BrdU index of β-cells was similarly lower in this group (0.13±0.04% vs 0.46±0.07%, p<0.005).

Conclusions: NN2211 ameliorated glycaemia and potentiated insulin secretion during OGTT in young pre-diabetic ZDF after 13 days of dosing. The basal levels of insulin and triglyceride were lower after NN2211, and body-weight gain was markedly reduced. However, the β-cell mass was lower than in the vehicle group, indicating that the powerful effects of NN2211 on insulin, triglycerides and body-weight were sufficient to control blood glucose resulting in a lesser need for an increased β-cell mass. Therefore NN2211 could be a promising drug for treatment of type 2 diabetic patients.

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NNC25-2504, A Potent Glucagon Receptor Antagonist

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Background and Aims: Increased hepatic glucose output is of major importance for the continued hyperglycaemia in type 2 diabetes, and glucagon antagonists have been discussed as a possible new therapy for years. In the absence of orally available compounds, intravenous administration of monoclonal antibodies against glucagon has been used successfully to validate the concept. Non-peptide glucagon antagonists have been reported, and one compound has also been in the clinic, but presently, no glucagon receptor antagonists seem to be in clinical trials. NNC25-2504 has an affinity for the cloned human glucagon receptor of 2.3 ± 0.6 nM (IC50). It has been shown that the compound has an acceptable kinetic profile, and that it can prevent glucagon induced hyperglycaemia in rats. We have observed large variations in the glucagon receptor binding affinity of non-peptide glucagon antagonists among different species, representing a problem when testing the compounds in different animal models. We have addressed the species differences using isolated liver plasma membranes. **Materials and Methods:** Livers were isolated from rats, mice, pigs, dogs and rabbits and plasma membranes were prepared. The human receptor was analyzed using plasma membranes from BHK cells expressing the receptor. Competition receptor binding assays and adenylate cyclase experiments were carried out using standard techniques. **Results:** The affinity (IC50) for the rat receptor was 0.43 ± 0.099 nM, the mouse receptor 0.51 ± 0.057 nM, the pig receptor 0.68 ± 0.11 nM, the dog receptor 1.1 ± 0.16 nM and the rabbit receptor 23 ± 6.7 nM. This compound was then characterized by a retained potency for most species, except the rabbit receptor. Thus, rabbits should be avoided as animal model for this type of compounds. When tested for its ability to antagonize the effect of glucagon on the cloned human receptor, the antagonist dose-dependently right-shifted the glucagon dose-response curve, but also lowered the maximal response thus behaving as a non-competitive antagonist. The KB was calculated to be 0.76 ± 0.18 nM. At the isolated rat liver receptor only the maximal response was lowered, NNC25-2504 behaving as a true non-competitive antagonist. The antagonist was selective for the glucagon receptor compared to the closely related GLP-1 receptor (IC50 406 ± 69 nM). In the presence of 2.5% albumin, the affinity for the human glucagon receptor was decreased to 69 ± 28 nM.

Conclusions: NNC25-2504 is a potent glucagon receptor antagonist that can be used for further validation of the concept of glucagon antagonism and maybe useful for the treatment of hyperglycemia in type 2 diabetes.

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BED-TIME ADMINISTRATION OF A NN2211, A LONG-ACTING GLP-1 DERIVATIVE, RESULTS IN A SUBSTANTIAL REDUCTION IN FASTING AND POSTPRANDIAL GLYCAEMIA IN TYPE 2 DIABETES

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Background and Aim: GLP-1 is a potent glucose-lowering agent of potential interest for the treatment of type 2 diabetes. The aim of this project was to evaluate the glucose lowering action of NN2211, a GLP-1 derivative, in type 2 diabetes. **Materials and Methods:** 11 patients with type 2 diabetes mellitus, age 59±7 years (mean±SD), BMI 28.9±3.0 kg/m², HbA1c 6.5±0.6% were examined in a double blind, placebo-controlled crossover design. A single sc. injection (10µg/kg) of NN2211 was administered at 11pm and profiles of circulating insulin, c-peptide, glucose and glucagon were obtained. A standardized mixed meal was served at 11.30am. Efficacy analyses were performed for the fasting (7-8am) and mealtime (11.30am – 3.30pm) periods. Glucose pulse entrainment (6mg/kg/min/10minutes, 9.30-10.30am) was evaluated by one-minute insulin samplings. Statistical analyses were performed by ANOVA.

Results	NN2211	Placebo
Fasting values		
plasma glucose (mM)	6.9±1.0	8.1±1.0 ‡
insulin secretory rate (pmol/min)	179±70	163±66 ‡
glucagon (pg/ml)	66±12	69±16
Meal related AUC_{11.30-13.30}		
plasma glucose (mmol/L4h)	7.7±0.6	10.0±1.8 ‡
insulin secretory rate (nmol)	118±32	106±27
glucagon (pg/ml4h)	67±16	71±15 ‡
gastric emptying, 3-OMG (mg/dl4h)	100±21	110±18 ‡
Glucose pulse entrainment		
spectral power	7.3±3.9	4.9±2.3 §
autocorrelation coefficient	0.16±0.13	0.11±0.17 §

‡ p<0.01, † p<0.05, § p<0.1, 3-OMG = 3-ortho methyl glucose

A favourable pharmacokinetic profile suitable for once daily dosing (t_{1/2}=10.0±3.5h, t_{max}=12.4±1.7h) was found. **Conclusion:** NN2211 effectively reduces fasting as well as meal-related plasma glucose by modifying insulin secretion, suppressing glucagon secretion and delaying gastric emptying.

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NN2211, A LONG-ACTING GLP-1 DERIVATIVE, DECREASES BLOOD GLUCOSE AND STIMULATES β -CELL PROLIFERATION IN DB/DB MICE
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Background and aims: NN2211 is a long-acting derivative of GLP-1 with kinetics suitable for once daily administration to humans. The present study compares the efficacy of NN2211 and exendin-4 (ex-4), a shorter acting GLP-1 analogue, on blood glucose (BG) and β -cell proliferation and mass in db/db mice, an animal model of type 2 diabetes. **Materials and methods:** 30 female diabetic db/db mice, 13 weeks old, received 200 μ g/kg NN2211, 100 μ g/kg ex-4, or vehicle s.c. twice daily (n=10). The ex-4 dose was slightly higher than the reported maximally effective dose in db/db mice. Both compounds were dosed twice daily because of more rapid metabolism in rodents. The 24-hour BG profile was measured in non-fasting mice on day 1, 8 and 15. On day 16 mice were given BrdU (100 mg/kg i.p.) before sacrifice. β -cell proliferation rate was estimated from incorporation of BrdU. β -cell mass was estimated stereologically. **Results:** Both NN2211 and ex-4 lowered AUC BG on day 1 and 15, but only NN2211 had significant effects on day 8 (Table 1). The effect of NN2211 was more marked than ex-4 on day 1 and 8. β cell proliferation rate increased from $0.42 \pm 0.05\%$ in vehicle to $1.27 \pm 0.16\%$ ($P < 0.001$ vs. veh., $P < 0.01$ vs. ex-4) and $0.68 \pm 0.11\%$ ($P < 0.05$ vs. veh.), in NN2211 and ex-4 groups respectively. β cell mass increased from 5.20 ± 0.66 mg to 8.54 ± 0.61 mg ($P < 0.01$ vs. veh., ns vs. ex-4) and 6.70 ± 0.96 mg (ns vs. veh.) in the NN2211 and ex-4 groups, respectively. **Conclusions:** NN2211 has a longer lasting blood glucose lowering effect than ex-4 following a twice-daily dosing schedule and increases β -cell mass by stimulating proliferation rate to a greater extent than ex-4 in the diabetic db/db mouse. This can be explained by the longer half-life of NN2211. Assuming that these data are predictive of clinical efficacy, NN2211 is a promising candidate for once daily treatment of type 2 diabetes

Table 1: Mean 24-hour AUC BG (mmol*hour)(data: Mean \pm SEM)

Day of dosing	Vehicle	NN2211	Exendin-4
1	389 \pm 16	193 \pm 24***	233 \pm 20*
8	415 \pm 11	278 \pm 28**	332 \pm 22
15	467 \pm 17	373 \pm 24*	366 \pm 18**

: Level of significance by Kruskal-Wallis test compared to vehicle

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Effect of the long-acting GLP-1 derivative NN2211 in 60 % pancreatectomized rats
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Background and Aims: NN2211 is a long-acting, metabolically stable GLP-1 derivative designed for once-daily administration. In addition to its other effects, GLP-1 may have a direct effect on the beta-cell mass. We have therefore investigated the effect of NN2211, in 60 % pancreatectomized rats. **Materials and Methods:** Male Sprague-Dawley rats (100 g) were subjected to 60 % pancreatectomy and allowed to recuperate for 4 days. Based on an OGTT at day 4 the rats were divided in three matched groups (n=8) and were injected s.c. twice daily with a) NN2211 (150 mg/kg/day), b) vehicle and pair-fed or c) vehicle from day 4 to day 8. At day 8 a second OGTT was performed. BrdU (100 mg/kg, i.p.) was administered 4 hours before sacrifice. Remnant and regenerated pancreas was removed from rats treated with two doses of NN2211 (100 or 150 microg/kg/day) or vehicle, and the tissue was immunostained for insulin and BrdU. **Results:** NN2211 treatment resulted in a significantly lowered area under the curve (deltaAUC0-120 min) in the OGTT at day 8 compared with both the pair-fed and vehicle treated groups. The deltaAUC0-120 min in the pair-fed vehicle-treated group was significantly higher than the NN2211 group and significantly lower than the vehicle treated group. Consequently the blood glucose lowering effect of NN2211 in the pancreatectomized rats is partly independent of the food-intake lowering effect of NN2211. Immunohistochemical staining for insulin points to an increase in beta-cell mass in the remnant pancreas of NN2211 treated animals, as evidenced by many considerably enlarged islets in this area. A semi-quantitative measurement revealed an approximately two-fold increase of the total beta-cell area in the NN2211-treated animals. There was no visible difference in the size of islets located in the regenerated area between the vehicle and NN2211 treated groups. Immunostaining for BrdU revealed a marked proliferation mainly in exocrine cells located at the border of the regeneration zones in both vehicle and NN2211-treated animals. At this timepoint (9 days after pancreatectomy) there was no effect of NN2211-treatment on the proliferative index in islets or in any other areas of remnant or regenerating pancreas. **Conclusions:** NN2211 lowers blood glucose after an oral glucose-load in 60% pancreatectomized rats, apparently due to an increase in the total beta-cell mass. The mechanism behind the increased beta-cell mass seen after NN2211-treatment appears to be proliferation or anti-apoptosis in the remnant islets rather than accelerated neogenesis in the zone of regeneration.

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GLP-1 EFFECT ON GLUCOSE TRANSPORT IN MYOCYTES FROM NORMAL AND TYPE 2 DIABETIC PATIENTS.

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Background and Aims: In rat and human myocytes, GLP-1 stimulates glycogen synthesis; in the skeletal muscle, GLP-1 was also shown to increase glucose transport in pieces of mouse tissue, and when infused in diabetic rats. In this work, we have studied, in primary cultured myocytes from normal and Type 2 diabetic patients, the action of GLP-1, and that of Ex-4 and Ex 9-39 (Ex-9) -both agonist of GLP-1 actions in L6 myoblast and 3T3-L1 adipocytes-, on glucose transport (GT), and that of GLP-1 on glycogen synthesis (Gly), compared with insulin. **Materials and Methods:** Myotubes were established from satellite cells of dissociated vastus lateralis, from 7 Type 2 diabetic patients (6F/2M; age: 81 ± 2 yr; fasting plasma glucose: 240 ± 24 mg/dl) and 2F normal subjects (age: 83 ± 4 yr; fasting plasma glucose: 132 ± 8 mg/dl), previous informed consent given, undergoing surgery; GT and Gly were measured in the absence (control) and presence of the peptides. **Results:** In normal subjects, 10^{-8} M GLP-1 increased GT [$48 \pm 13\%$ of control (11.1 ± 1.1 pmol/2x10⁴ cells in 5 min, n=4), n=4, $p < 0.05$], although in a lower magnitude ($p < 0.05$) than that induced by 10^{-8} M insulin ($142 \pm 31\%$, n=6, $p < 0.01$); when insulin was tested in combination with GLP-1, no further increase was detected; 10^{-8} M Ex-4 ($62 \pm 12\%$, n=6) and 10^{-8} M Ex-9 ($94 \pm 18\%$, n=5) both produced a similar increment ($p < 0.02$ and $p < 0.01$, respectively) to that exerted by GLP-1. In Type 2 diabetics, while 10^{-8} M insulin apparently failed to affect GT, 10^{-8} M GLP-1 [$96 \pm 20\%$ of control (8.4 ± 1.6 pmol/2x10⁴ cells, n=7), n=4, $p < 0.05$], 10^{-8} M Ex-4 ($90 \pm 27\%$, n=6) and 10^{-8} M Ex-9 ($78 \pm 28\%$, n=6), significantly ($p < 0.05$ or lower) increased GT, to the same extent as in normal subjects; the respective increments on Gly by 10^{-16} M insulin [$14 \pm 2\%$ of control (10.5 ± 0.9 nmol/mg, n=5), n=5, $p < 0.02$] or 10^{-8} M ($19 \pm 3\%$, n=5, $p < 0.05$), and by 10^{-16} M GLP-1 ($18 \pm 2\%$, n=5, $p < 0.02$) or 10^{-8} M ($24 \pm 4\%$, n=5, $p < 0.05$), were reduced ($p < 0.05$ or lower) respect those previously observed in normal subjects. **Conclusions:** GLP-1 equally increases GT in myocytes from normal as well as from Type 2 diabetic subjects, supporting its proposed therapeutic use. The similar increasing action of both exendins on GT reinforces the view of a muscle GLP-1 receptor different from the pancreatic one.

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Exendin-4 Injections: Ameliorated Progression of Fasting Hyperglycemia, Elevation of Fructosamine HbA1c, Total Cholesterol and Increased C-peptide Level in Prediabetic Zucker Diabetic Fatty Rats

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Background and Aims: Exendin-4 (AC2993), a 39 amino acid peptide, is reported to have a number of antidiabetic actions that include glucose-dependent stimulation of insulin secretion, modulation of nutrient assimilation and inhibition of glucagon secretion. Diabetes is commonly associated with various lipid disorders, which may be primary or secondary to diabetes. The purpose of the present study was to evaluate the effect of exendin-4 injections on the progression of diabetes in prediabetic Zucker diabetic fatty (ZDF) rats.

Materials and Methods: Beginning at age 7 weeks, rats were injected twice daily for 8 further weeks with saline (n=6), twice daily with 1 μ g exendin-4 (n=7), or once daily with 1 μ g exendin-4 (n=4). Fasting plasma glucose, fructosamine, HbA1c, total cholesterol concentrations were measured from tail blood samples every 2 weeks and C-peptide level after 8 weeks of studies.

Results: In saline-treated control rats, fasting plasma glucose increased by $278.8 \pm 10.5\%$ over 8 weeks, HbA1c by 6.2 ± 0.46 percent units, fructosamine from 147.3 ± 1.2 μ mol/L to 226.6 ± 6.5 μ mol/L, and plasma total cholesterol from 106.2 ± 4.6 mg/dL to 226.8 ± 31.4 mg/dL. In contrast, rats injected twice daily with exendin-4 showed increase in fasting glucose of $72.4 \pm 8.5\%$ of pretreatment values ($P < 0.001$ vs control), and an increase in HbA1c of 1.01 ± 0.32 percent units ($P < 0.001$ vs control), no increase in plasma fructosamine level 149.1 ± 1.3 μ mol/L vs 149.3 ± 6.13 μ mol/L ($P < 0.001$ for increment vs control), and a lesser increase in fasting total cholesterol (from 109.6 ± 3.5 to 163.3 ± 5.4 mg/L) than did saline controls ($P < 0.001$). Reduction in these measures of diabetes progression was intermediate between these responses with once daily exendin-4 injections. Respective C-peptide levels after 8 weeks, for saline, once daily and twice daily treatment were 688.5 ± 80.2 , 1586.1 ± 205.6 , 2983.4 ± 541.5 pM ($P < 0.001$, $P < 0.001$ vs saline controls).

Conclusions: In this ZDF rat model of type 2 diabetes, exendin-4 reduced measures of progression of diabetes and ameliorated the associated hypercholesterolemia.

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Continuous Infusion of Exendin-4 Lowered HbA1c at Least as Effectively as Twice Daily Injections in Diabetic Fatty Zucker (ZDF) Rats.

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Background and Aims: In previously reported studies done in Diabetic Fatty Zucker (ZDF) rats, daily peripheral injections of synthetic exendin-4 (AC2993) lowered hemoglobin A1c (HbA1c). In rats, HbA1c is a useful index of blood glucose averaged over ~4 weeks. Some hormones are only fully effective if delivered in a pulsatile manner. The purpose of the present study was to compare the effect on HbA1c of continuous infusion of exendin-4 versus the same dose delivered by twice daily injection, and thus determine if continuously present exendin-4 was associated with a diminished glycemic effect.

Materials and Methods: Three groups of animals (n=5/group) were each implanted with Alza pumps (delivering 60µL/24 hours) and were injected i.p. twice daily (30µL/injection). Control animals (CONT) received vehicle by pump and i.p. injection; continuous infusion (INF) animals received exendin-4 via Alza pumps (18µg/60µL/24 hours) and vehicle via twice daily i.p. injections; the pulsatile injection (INJ) group received the same dose via two 9µg/30µL/injections each day for 28 days. HbA1c was measured weekly.

Results: Entry HbA1c of the 3 treatment groups were indistinguishable (range 9.30-9.38%; n.s.). After 28 days of treatment, HbA1c had fallen from 9.32±0.46 to 8.70±0.33% in CONT (n.s.), from 9.30±0.37 to 7.74±0.63 in INJ (P=0.07), and from 9.38±0.41 to 5.64±0.76% in INF (P=0.003). Thus, after 28 days of treatment, the mean decrement in HbA1c of the INF group (3.74±0.48) was greater than that of the INJ group (1.56±0.93, P=0.07).

Conclusions: Continuous infusion of exendin-4 was effective in lowering HbA1c in ZDF rats, and this effect was at least as great as that of the same dose given as a twice-daily injection. Thus, in this experimental model of type 2 diabetes, hormonal action of exendin-4 was not diminished by its continual presence.

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LIPOLYTIC EFFECT OF GLP-1 IN ISOLATED ADIPOCYTES FROM MORBIDLY OBESE SUBJECTS.

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Background and Aims: GLP-1, at doses higher than 10^{-10} M, exerts in rat and normal human adipocytes a concentration-related lipolytic effect. In this work, we have studied the effect of 10^{-9} M GLP-1 on lipolysis, in isolated adipocytes from morbidly obese patients, as well as that of glucagon and insulin. **Materials and Methods:** Adipocytes were isolated by enzymatic digestion from subcutaneous fat tissue, obtained, previous informed consent given, from six morbidly obese female patients undergoing bariatric surgery (age: 48±2 yr; BMI: 54±3 kg/m²; fasting plasma glucose: 115±14 mg/dl; cholesterol: 202±16 mg/dl; triglycerides: 146±20 mg/dl; HDL: 43±1 mg/dl). Lipolysis was measured as the glycerol released by 10^5 cells, incubated for two hours in the absence (control) and presence of 10^{-9} M GLP-1 or glucagon, and without or with 10^{-9} M insulin. Data are presented as mean±SEM; the statistical significance of difference between mean values was assessed by Student's *t*-test. **Results:** In obese patients, 10^{-9} M GLP-1 induced a highly significant increase in glycerol release [83±10%Δ of control (17.4±1.5 nmol/ 10^5 cell), n=6, p<0.001], which was of the same magnitude as that induced by 10^{-9} M glucagon (89±7%Δ, n=6, p<0.001), being both higher (p<0.05 or lower) than that previously observed in five normal individuals of similar age (43±14%Δ and 45±12%Δ, respectively); 10^{-9} M insulin significantly decreased the lipolysis control value (-38±7%Δ, n=5, p<0.01), and also abolished the stimulus induced by either GLP-1 (-32±7%Δ, n=5, p<0.01, and p<0.001 vs GLP-1) or glucagon (-40±7%Δ, n=5, p<0.01, and p<0.001 vs glucagon). **Conclusions:** In isolated adipocytes from morbidly obese patients, GLP-1, at 10^{-9} M, produced a lipolytic effect, similar to that of the equimolar dose of glucagon, and apparently higher than in those from normal subjects. The effect of both peptides was abolished by the simultaneous presence of the equimolar dose of insulin.

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EXENDIN-4[1-39] INCREASES INSULIN-MEDIATED GLUCOSE UPTAKE IN L6 & 3T3 CELLS VIA A PI-3-KINASE-DEPENDENT MECHANISM

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Background and Aims: The primary antidiabetic mechanism of glucagon-like peptide-1 (GLP-1) and its long-acting analogue, exendin-4[1-39], involves stimulation of pancreatic insulin secretion, but additional metabolic effects have not been fully investigated.

Methods: The effects of exendin-4 on insulin-stimulated 2-³H]deoxyglucose (2DOG) uptake were examined in fully differentiated L6 myotubes and 3T3-adipocytes. Cells were incubated with insulin 100nM and varying concentrations of exendin-4 (1-100nM) or GLP-1, and separate co-incubations with wortmannin 100nM (a PI-3-kinase inhibitor) and PD098059 (a MAP-kinase inhibitor) were performed.

Results: In L6 myotubes, both GLP-1 and exendin-4 significantly augmented insulin-stimulated glucose uptake: eg, uptake was 100% (basal) vs 126% (insulin) and 171% (insulin + GLP-1), p<0.0001; and correspondingly 100% (basal) vs 110% (insulin) and 134% (insulin + exendin-4), p<0.05. The insulin-sensitizing effect of exendin-4 was abolished by wortmannin and PD098059. In contrast, GLP-1 had no effect on 2DOG uptake in 3T3-adipocytes, whereas exendin-4 enhanced insulin sensitivity via a pathway inhibited by wortmannin but not by PD098059.

Conclusions: Thus, GLP-1 increases insulin sensitivity in L6 myotubes, but not 3T3-adipocytes, whereas exendin-4 enhances insulin-stimulated glucose uptake in both cell types. The insulin-sensitizing effects of these incretin molecules are PI-3-kinase-dependent.

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Recombinant glucagon-like peptide-1 lowers fasting plasma glucose in a broad spectrum of type 2 diabetes

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Background and Aims: Glucagon-like peptide-1 (7-36)amide (GLP-1) has great promise as a novel therapeutic for type 2 diabetes. Clinical development of the natural peptide has been hampered, however, by its high cost and the lack of a clinically useful formulation and delivery system. We administered recombinant GLP-1 (rGLP-1) subcutaneously (sc) to 40 type 2 diabetes in a double-blind, placebo-controlled, cross-over trial, in which we evaluated the efficacy of injection versus infusion and the response of early- to late-stage patients.

Materials and Methods: Four groups of 10 patients were recruited: (1) diet, (2) sulfonylurea (SU), (3) metformin (MET), and (4) insulin; pre-existing treatment was not withdrawn. Prior to randomization, all patients received two sequential bolus injections of rGLP-1 (1.5 nmol/kg) 2 hours apart after a standard meal, and were followed for 4 hours. After the test day, each patient received several doses of rGLP-1 in random order by two routes: (a) injection phase: 2 injections (0, 0.5, 1.0, 1.5 nmol/kg/injection) after the evening meal; (b) infusion phase: continuous sc infusion of rGLP-1 (0, 1.5, 2.5, 3.5, 4.5 pmol/kg/min) for 12 hours overnight starting after the evening meal.

Results: During the test day, all patients, including the insulin cohort, showed increased insulin and decreased glucagon levels and concomitant reductions in plasma glucose levels following the rGLP-1 injections. The diet, SU, and MET cohorts showed modest (15 mg/dL) reductions in fasting plasma glucose (FPG) levels (p<0.05) during the injection phase, and dose-dependent reductions in FPG (up to 44 mg/dL, p<0.001) during the infusion phase. The insulin cohort showed a significant reduction in FPG (29 mg/dL, p=0.004) at the highest infusion dose. rGLP-1 also dose-dependently reduced fasting plasma glucagon (p<0.05) and increased fasting plasma insulin (p<0.03) levels. No serious or unexpected adverse events were recorded; there was one case of biochemical hypoglycemia (44 mg/dL). GI-related symptoms were dose-related and 81% associated with injections.

Conclusions: We conclude that rGLP-1 effectively and dose-dependently lowers FPG in a broad spectrum of type 2 diabetic patients when administered by continuous sc infusion.

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Thiazolidinediones/Lipids

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Prevention of restenosis after balloon injury of carotid artery by troglitazone in OLETF rats

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Background and Aims: Thiazolidinediones (TZD) are a new class of antidiabetic agents which act through binding to a nuclear receptor peroxisome proliferator-activated receptor- γ (PPAR- γ). PPAR- γ has been known to be involved in chronic inflammation and atherosclerosis. Most of the actions of PPAR- γ are preventive but it also facilitated the uptake of cholesterol by scavenger receptors and promoted the foam cell formation. We performed this study to investigate the effects of PPAR- γ ligand, TGZ, on the restenosis after balloon injury of carotid artery in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a type 2 diabetic animal model.

Materials and Methods: Male OLETF rats (n=20) were divided into 2 groups. Group 1 (n=10) was fed with normal rodent chow and group 2 (n=10) was fed with TGZ as 0.15% food admixture ad libitum from 16 weeks of age. 1 week later, balloon injuries were made to left carotid arteries of 2 groups of rats and 2 weeks after balloon injury, animals were sacrificed after taking blood samples and Doppler ultrasonography. Histomorphometry of the carotid arteries was done after H & E staining.

Results: Fasting plasma glucose level was not significantly different between 2 groups (23.6 \pm 2.3 vs 24.5 \pm 2.8 mmol/L). Plasma insulin level was significantly higher in group 1 compared to group 2 (1.9 \pm 0.3 vs 1.3 \pm 0.3 ng/ml, p<0.05). Plasma total cholesterol level was higher in group 1 compared to group 2 (198 \pm 29 vs 97 \pm 32 mg/dl, p<0.0001) and so was triglyceride level (210 \pm 32 vs 87 \pm 29 mg/dl, p<0.0001). Blood flow velocity measured by Doppler echocardiography was significantly decreased in left carotid arteries of group 1 compared to that of group 2 (0.65 \pm 0.08 vs 0.91 \pm 0.09 m/sec, p=0.0004). Neointimal area of left carotid arteries measured by histomorphometry was significantly decreased in group 2 compared to group 1 (0.19 \pm 0.02 vs 0.13 \pm 0.02 mm², p=0.002). Neointima/media ratio was also significantly decreased in group 2 compared to group 1 (1.7 \pm 0.2 vs 1.2 \pm 0.2, p=0.0004). TGZ, when directly incubated with rat vascular smooth muscle cells in vitro, significantly decreased insulin-stimulated proliferation at concentrations of 10 and 20 μ g/ml (p<0.05 vs 100 ng/ml insulin stimulation).

Conclusions: These results suggest that PPAR- γ ligands might prevent the restenosis after balloon injury of carotid arteries in type 2 diabetic animal models.

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ROSIGLITAZONE REDUCES MYOCARDIAL INFARCTION AND IMPROVES CONTRACTILE DYSFUNCTION IN AN IN VIVO RAT ISCHAEMIA/REPERFUSION INJURY MODEL

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Background and Aims: Diabetes is associated with increased risk of mortality as a consequence of acute myocardial infarction. This study determined whether rosiglitazone (RSG), a new thiazolidinedione antidiabetic agent, could reduce myocardial infarction after ischaemia/reperfusion injury.

Materials and Methods: Male Lewis rats were anaesthetised, and the left anterior descending coronary artery ligated for 30 minutes. Following reperfusion for 24 hours, the ischaemic and infarct sizes were determined.

Results: RSG at 1 and 3 mg/kg given intravenously, half the dosage prior to ischaemia and half following reperfusion, reduced infarct size by 30% and 37%, respectively (p<0.01 vs vehicle). Pretreatment with RSG (3 mg/kg/day) orally for 7 days also reduced infarct size by 24% (p<0.01). RSG also improved ischaemia/reperfusion-induced myocardial contractile dysfunction. Left ventricular (LV) systolic pressure, and positive and negative maximal values of the first derivative of LV pressure (+dp/dt and -dp/dt) were significantly improved in RSG-treated rats. RSG reduced the accumulation of neutrophils and macrophages in the ischaemic heart by 40% and 43%, respectively (p<0.01). Ischaemia/reperfusion induced upregulation of CD11b/CD18 and downregulation of L-selectin on neutrophils and monocytes; these effects were significantly attenuated in RSG-treated animals. Likewise, intercellular adhesion molecule-1 expression in ischaemic hearts was markedly diminished by RSG, as was the ischaemia/reperfusion-stimulated upregulation of monocyte chemoattractant protein-1.

Conclusions: The present study suggests that the cardioprotective effect of RSG might be due to inhibition of the inflammatory response. The cardioprotective effect of RSG would benefit the diabetic patients for whom myocardial dysfunction is a common life-threatening complication.

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Rosiglitazone prevents ischaemic injury in the obese Zucker rat heart

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Background and Aims: Type II diabetic patients have higher mortality than non-diabetics post myocardial infarction. Rosiglitazone (RSG) is a PPAR γ activator that decreases plasma fatty acid concentrations and improves tissue insulin sensitivity. As high fatty acids increase ischaemic injury, we hypothesized that RSG would be cardioprotective in obese Zucker (OZ) rat, a highly dyslipidemic animal model.

Materials and methods: Lean Zucker (LZ) and OZ rats (12 mo) were treated for 7-14 d with RSG 3 mg/kg p.o. or vehicle (n = 7 per group). Hearts were perfused ex vivo with buffer (11 mM glucose) and subjected to low-flow ischaemia (0.5 ml/min/gww) followed by reperfusion. ³H-glucose was used as a tracer of glucose utilization during ischaemia and ³¹P NMR spectroscopy was used to follow myocardial pH and energetics. Total GLUT-4 heart content was determined by Western blotting.

Results: Initial contractile function was the same for all hearts. At reperfusion, the final recovery of control OZ hearts was (mean \pm SEM) 52 \pm 10% of initial contractile function, significantly lower than RSG-treated OZ hearts (81 \pm 7%; p < 0.05) or LZ controls. LZ hearts were unaffected by RSG. The reduced recovery of OZ hearts was associated with fibrillation (86% vs 0% in LZ hearts). RSG feeding reduced the incidence of fibrillation in OZ hearts (26%). Glucose uptake during ischaemia was lower in control OZ hearts than control LZ hearts (11.6 \pm 0.7 vs 14.7 \pm 0.3 mmol; p < 0.05), but was normalized by RSG (14.8 \pm 0.7 mmol). Total GLUT-4 content in OZ hearts was 30 \pm 11% lower than in LZ hearts, and was restored by RSG treatment. Loss of cytosolic ATP during ischaemia was greater in control OZ hearts (-0.11 \pm 0.02 mmol/gww/min) than RSG-OZ hearts (-0.03 \pm 0.06 mmol/gww/min; p < 0.05), leading to a 50% lower end-ischaemic ATP. End-ischaemic pH was also significantly lower in control than in RSG-treated OZ hearts (6.31 \pm 0.06 vs 6.63 \pm 0.09; p<0.05).

Conclusions: RSG prevented the increased ischaemic injury seen in OZ hearts, probably by normalizing glucose uptake during ischaemia and thereby increasing glycolytic ATP production, limiting ATP loss and fall in pH. This work indicates that PPAR γ may have cardioprotective properties for the OZ rat heart and a potentially important effect of RSG.

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COMPARATIVE EFFECTS OF THIAZOLIDINEDIONES ON PLASMA GLUCOSE AND TRIGLYCERIDES IN A MOUSE MODEL OF DIABETES

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Background and Aims: The effects of the PPAR α/γ modulator RWJ 241947/MCC-555, troglitazone, pioglitazone, and rosiglitazone on plasma glucose, triglycerides (TG), and HbA_{1c} were compared. **Materials and Methods:** Drugs were administered orally for 8 weeks to db/db mice, a model of type 2 diabetes. **Results:** At the highest dose, RWJ241947, a novel insulin sensitizer with modulatory activity at PPAR α and PPAR γ , decreased plasma glucose and TG in a time- and dose-dependent manner, to about the level of db/+ mice. Troglitazone lowered glucose but the effect was not significant or dose dependent. Pioglitazone decreased glucose significantly. Rosiglitazone lowered glucose and TG significantly. RWJ241947 reduced HbA_{1c} in a dose-dependent manner, and the decreases were significant at 10 and 30 mg/kg (p<0.01). Rosiglitazone also lowered HbA_{1c} significantly (p<0.01). Troglitazone and pioglitazone did not decrease HbA_{1c} significantly.

Weight gain, plasma glucose and triglycerides after 8 weeks

	Weight gain (g)	Glucose (mg/dL)	TG (mg/dL)
db/+	4.8	207.4	136.2
db/db control	3.9	754.6	280.5
RWJ241947 1 mg/kg/d	2.7	711.3	310.6
RWJ241947 3 mg/kg/d	11.4	565.1	170.5
RWJ241947 10 mg/kg/d	18.3**	487.3*	117.7
RWJ241947 30 mg/kg/d	28.1**	175.5**	89.4*
Troglitazone 200 mg/kg/d	11.0	607.7	115.6
Troglitazone 400 mg/kg/d	11.3	631.1	123.7
Pioglitazone 30 mg/kg/d	15.4**	552.1*	148.5
Rosiglitazone 30 mg/kg/d	24.5**	231.9**	48.4**

* p<0.05; ** p<0.01 versus db/db control

Conclusions: The PPAR α and PPAR γ modulator, RWJ241947, at 30 mg/kg/d, and rosiglitazone 30 mg/kg/d effectively lowered plasma glucose and TG.

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RAPID REVERSAL OF HEPATIC STEATOSIS BY ROSIGLITAZONE

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Background and Aims: Obesity with or without Type 2 diabetes is frequently associated with hepatic steatosis, which may pre-dispose to serious liver injury. Rosiglitazone (RSG) reduces hepatic steatosis in humans, though the time to onset is unknown.

Materials and Methods: Zucker fatty (ZF) rats (*fa/fa*), aged 12 weeks (*n*=8/group), were given RSG (3 mg/kg/day p.o.) (ZF-RSG) or placebo (ZF-CON) for 13 weeks at matched levels of food intake, and repeat proton-magnetic resonance spectroscopy was used to monitor time to, and durability of, effect on hepatic steatosis. Zucker lean (ZL) rats (*+/+*) served as normal controls (*n*=8). Plasma insulin and lipids were measured serially.

Results: Very low levels of liver fat (<1%) were present in ZL rats throughout, but the fat:water ratio (%; mean±SD) in ZF-CON rats was persistently elevated: 10.6±3.3 (week -1), 15.0±3.4 (week +2), 13.4±5.3 (week +4), 11.4±3.2 (week +9), 14.8±5.8 (week +13). In ZF-RSG rats, this ratio fell from 7.8±2.2 at week -1 (*p*>0.05 vs ZF-CON) to 3.1±1.1 (*p*<0.0005 vs ZF-CON for change from week -1), 2.3±0.7 (*p*<0.05), 2.7±0.8 (*p*>0.05) and 3.7±1.4 (*p*<0.01) at week +2, 4, 9 and 13, respectively. Further, liver volume (ml) escalated in ZF-CON rats (from 20.7±1.6 at week -1 to 24.3±1.3 at week +13) but fell in ZF-RSG rats (from 19.2±2.2 at week -1 to 17.8±1.3 at week +13; *p*<0.05 vs ZF-CON for change from week -1). The RSG-mediated reductions in hepatic fat/volume coincided with reduced hyperinsulinaemia, but were inconsistent with changes in plasma lipids. For plasma insulin (mean±SD; ng/ml), ZF-CON values were maintained at 60.0±19.2 (week -1), 53.9±17.0 (week +4), 65.0±30.3 (week +9) and 59.6±16.3 (week +13). In ZF-RSG rats, plasma insulin fell from 38.8±18.7 (week -1) to 4.2±1.2 (week +4; *p*<0.05 vs ZF-CON for change from week -1), 12.8±3.9 (week +9; *p*<0.05) and 7.8±2.5 (week +13; *p*<0.02).

Conclusions: RSG rapidly and durably reverses hepatic steatosis and hepatomegaly in ZF rats. This effect occurs with improved insulinaemia but is seemingly independent of plasma lipids, perhaps suggesting a direct mobilising effect of RSG on liver fat.

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Up-regulation of mitochondrial fatty acid oxidation pathway after PGZ treatment may prevent tissue TG accumulation and improve insulin sensitivity in HF diet-induced IR.

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Background and Aims: Pioglitazone (PGZ), the newly approved insulin sensitizing thiazolidinedione, has also a hypolipidemic potential, which is still under investigation. The aim of this study was to analyze the possible mechanisms responsible for its beneficial effect on lipid metabolism in the high fat (HF) diet-induced insulin resistance (IR). **Material and Methods:** Male Wistar rats were fed for 21 days a HF diet (70 cal%). A half of them was simultaneously treated with PGZ (6 mg per kg BW once daily by gavage). Rats fed a standard lab chow were used as control (C). Triglyceride (TG), non-esterified fatty acids (NEFA), glycerol and insulin levels in serum, and tissue TG content were determined using commercial kits. Hepatic and muscular β -oxidation rate and activities of the key enzymes of mitochondrial (carnitine palmitoyl-transferase-I /CPT-I/) and peroxisomal (/AOX/ acyl-CoA oxidase) fatty acid oxidation were determined radiometrically.

Results: Feeding rats the HF diet led to hypertriglyceridemia (C: 2.0±0.2 vs. HF: 5.0±0.8 mM) and elevated liver (C: 6.4±1.1 vs. HF: 16.0±3.0 μ mol.g⁻¹) and skeletal muscle (C: 2.4±0.4 vs. 7.7±1.2) TG content. PGZ treatment normalized all blood and tissue lipids, and the *in vivo* insulin action (as assessed by euglycemic hyperinsulinemic clamp) as well. These effects were associated with an increase of hepatic (HF: 178±20 vs. HF+PGZ: 448±24 pmol.mg⁻¹.min⁻¹, *p*<0.001) and skeletal muscle (HF: 130±13 vs. HF+PGZ: 212±15, *p*<0.005) β -oxidation. Measurements of enzyme activities in skeletal muscle revealed that PGZ stimulates mitochondrial (CPT-I, HF: 450±50 vs. HF+PGZ: 720±110 pmol.mg⁻¹.min⁻¹, *p*<0.05), but not the peroxisomal (AOX, HF: 28.3±4.5 vs. HF+PGZ: 24.3±2.4 pmol.mg⁻¹.min⁻¹, *p*=NS) fatty acid oxidation. No changes in activities of CPT-I or AOX were found in the liver. Tissue lipid content correlated negatively with β -oxidation in both liver (*r*= -0.75, *n*=17, *p*<0.001) and muscle (*r*= -0.71, *n*=17, *p*<0.001). **Conclusions:** Our data indicates that an up-regulation of the β -oxidation pathway after PGZ treatment may prevent the tissue TG accumulation in the HF diet-induced IR. It is likely that the mitochondrial fatty acid oxidation could be one of the important targets for pioglitazone action.

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Thiazolidinediones: Treatment

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ROSIGLITAZONE TREATMENT OF PATIENTS WITH SEVERE PRIMARY INSULIN RESISTANCE AND DIABETES MELLITUS HAS NO EFFECTS ON GLUCOSE AND LIPID METABOLISM. H. Vestergaard¹, S. Lund² and O. Pedersen³.

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Background and Aims: Rosiglitazone (Avandia) increases insulin sensitivity by reducing levels of p-NEFA, triglycerides (TG), glucose and insulin. Rosiglitazone treatment decreases insulin resistance in type 2 diabetic patients, but no data exist concerning rosiglitazone treatment of patients with syndromes of severe insulin resistance. To evaluate whether hyperglycaemia in two patients with primary severe insulin resistance and diabetes mellitus could be reduced by supplement of rosiglitazone and secondary, to evaluate the effects on p-NEFA, TG, Apo B, PAI-1 and insulin. **Materials:** Both patients (brothers) have known mutations in the insulin receptor (IR) gene localized to the tyrosine kinase domain and a deletion of exon 17 in part of their IR mRNA. Prior to the study the HbA_{1c}>10% in both patients for more than 12 months during treatment with regular insulin (BC: 3.5 U/kg/day; KC: 4.7 U/kg/day) and metformin (1500mg/day). **Results:** After 180 days of max. rosiglitazone supplement (8 mg/day), no changes were observed in fasting p-glucose (BC: 11.7, 13.6, 11.5 and 11.9 mM day 0, 45, 90 and 180; KC: 15.5, 13.1, 15.7 and 15.4 mM day 0, 45, 90 and 180) and HbA_{1c} (BC: 10.9%, 11.2%, 10.8%, 11.6% day 0, 45, 90 and 180; KC: 11.2%, 11.4%, 11.1%, 11.3% day 0, 45, 90 and 180). Incremental p-glucose areas under the curves during a 75 g OGTT were unchanged (BC: 2492, 2532 and 2643 mM x 240 min, day 0, 45 and 180; KC: 1594, 1472 and 1448 mM x 240 min, day 0, 45 and 180). Likewise, no improvements were seen in either 1. or 2. phase insulin secretion during an 0.3 g/kg IVGTT. Fasting p-cholesterol (total, LDL- and HDL), TG and Apo B levels were unchanged. Fasting p-NEFA increased 51% in KC after 90 days of treatment, and after 180 days p-NEFA was still 26% higher, when compared to pretreatment levels. In BC an initial 16% decrease was seen in p-NEFA after 90 days of treatment. Plasma NEFA was increased 14% after 180 days of treatment, when compared to pretreatment levels, but 35% when compared to day 90. Plasma PAI-1 decreased in both patients after 45 and 90 days of treatment but the decrease was maintained only in KC (47%). **Conclusions:** Rosiglitazone treatment, in combination with insulin and metformin, of patients with severe primary insulin resistance and diabetes mellitus, has no effects on glucose and lipid metabolism. Rosiglitazone may reduce the cardiovascular risk in the patients by reducing PAI-1.

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ROSIGLITAZONE IMPROVES INSULIN SENSITIVITY AND 24-HOUR AMBULATORY BLOOD PRESSURE IN SUBJECTS WITH IGT

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Background and Aims: Rosiglitazone (RSG) improves insulin sensitivity and reduces ambulatory blood pressure (ABP) in Type 2 diabetics, but has not previously been studied in subjects with IGT. **Materials and Methods:** In a double-blind study, 18 subjects with persistent IGT were randomised to receive RSG (4 mg twice daily) or placebo for 12 weeks. Euglycaemic hyperinsulinaemic clamp was used to derive the insulin sensitivity index (ISI), and 24-hour ABP was monitored. **Results:** Baseline systolic and diastolic ABP were higher in the RSG group, and there was a trend to greater insulin sensitivity in the placebo group. RSG significantly improved ISI compared with baseline (BL, *p*=0.025, mean 36% increase) and placebo (*p*=0.0003), and significantly reduced systolic and diastolic ABP vs BL and vs placebo. Data below are mean or, for change from baseline, mean ± SD:

		Placebo (<i>n</i> =9)	RSG (<i>n</i> =8)†
ISI (μ g/kg/min/pmol/l)	BL	9.94	7.09
	Change vs BL	-1.82 ± 4.48	1.72* ± 1.72
	Change vs placebo	-	2.26**
24-hour ABP, systolic (mmHg)	BL	120.8	132.0
	Change vs BL	2.7 ± 5.7	-7.0** ± 4.8
	Change vs placebo	-	-9.8**
24-hour ABP, diastolic (mmHg)	BL	68.8	75.9
	Change vs BL	2.6 ± 5.5	-6.4* ± 5.2
	Change vs placebo	-	-8.0*

p*<0.05; *p*<0.01; †8 subjects completed, one lost to follow-up.

Conclusions: Consistent with its Type 2 diabetes effects, RSG substantially improves insulin sensitivity and reduces systolic and diastolic ABP in IGT.

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MECHANISMS BY WHICH ROSIGLITAZONE IMPROVES GLYCAEMIC CONTROL IN TYPE 2 DIABETES

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Background and Aims: The mechanisms by which thiazolidinediones (TZDs) improve glycaemic control are poorly understood. It is not clear whether TZDs act principally via decreasing glucose production, improving glucose disposal, or solely by improving insulin sensitivity. **Materials and Methods:** We studied various aspects of fasting (F) and postprandial (PP) glucose and lipid metabolism in 30 subjects with Type 2 diabetes before and after randomisation to 3 months' treatment with placebo (PBO, n=15) or rosiglitazone (RSG, 4 mg twice daily, n=15). **Results:** Compared with the PBO group, RSG responders (RR, ↓ in HbA_{1c} >0.3% or ↓ in F glucose >10%, n=12) decreased their HbA_{1c}, F and mean PP plasma glucose by 1.0%, 40 mg/dl and 43 mg/dl respectively (all p<0.03). Both F and PP plasma free fatty acids also decreased in RR (both p= 0.011). Insulin sensitivity (IS) and beta-cell function (β-fn) (HOMA) improved, in RR only, by 32±5% (p=0.004) and 27±8% (p=0.026), respectively. Similarly, F and PP endogenous glucose production (double-isotope technique) decreased in RR (p=0.01 and 0.023) whereas none of these parameters changed in the PBO group. PP but not F gluconeogenesis (¹⁴C-bicarbonate) decreased in RR (p=0.033, one tailed). Neither F nor PP tissue glucose uptake and oxidation (calorimetry) were altered in RR, but both F and PP glucose clearance increased (p=0.04 and 0.007). F and PP HDL-cholesterol increased significantly in RR (p=0.039 and 0.014); neither triglycerides nor LDL-cholesterol changed. **Conclusions:** In responsive patients with Type 2 diabetes, RSG improves glycaemic control by increasing both IS and β-fn. The actions of RSG primarily result in glycogenolytic and gluconeogenic reductions in glucose production and, to a lesser extent, more efficient glucose disposal.

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Long-term Insulin and Rosiglitazone Mediated Regulation of LPL, HSL & Lipolysis in Human Adipose Tissue

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Background and Aims: Lipolysis is an important process determining fuel metabolism and insulin regulates this process in adipose tissue. The aim of this study was to investigate the long-term effects of insulin (1-1000nM), an insulin enhancer (rosiglitazone: RSG), and both in combination, on the regulation of lipolysis and lipogenesis in human abdominal subcutaneous fat.

Materials and Methods: Lipolysis and lipogenesis were assessed by Western analysis of hormone sensitive lipase (HSL) and lipoprotein lipase (LPL) respectively. Lipolytic activity was assessed by glycerol release assay and tumour necrosis factor alpha (TNF-alpha) by ELISA (n=12).

Results: In subcutaneous adipocytes increasing insulin (Ins) stimulated LPL expression with maximal stimulation at 100nM ((Control: 1.00±0.0 (mean ± SE, protein expression relative to control) Ins 1nM: 0.87±0.13; Ins 100nM: 1.68±0.19***; Ins 1000nM: 1.4±0.07; ***p<0.001). In contrast, insulin altered HSL expression in an opposite pattern to LPL, with maximal reduction of HSL observed at 100nM insulin treatment (Control 1.00±0.0; Ins 100nM: 0.49±0.05*, *p<0.05). However, higher insulin doses (500nM-1000nM) stimulated both LPL and HSL expression (p<0.05). The addition of RSG induced a shift in response to the left in the dose response to insulin for LPL (Ins 1nM: 1.09±0.36, Ins 10nM 2.26±0.48* Ins 100nM 1.8±0.3*; Ins 1000nM: 1.18±0.13) and HSL (Ins 1nM: 2.92±0.48; Ins 10nM: 1.4±0.25; Ins 1000nM 4.48±1.32*; Ins 1000nM: 7.10±2.09*). In adipocytes treated with RSG alone there was a dose-dependent increase in LPL expression (RSG 10-5M: 11.20±2.42*; RSG10-10M: 6.28±1.43*) but a dose-dependent decrease in HSL expression (RSG10-6M: 2.13±0.16***; RSG 10-10M: 9.07±1.79**; **p<0.01). High levels of insulin stimulated glycerol release (Control: 244.3±21μmol; Ins 100nM: 364.3±52μmol*; Ins 1000nM: 493.7±80μmol**) correlating with HSL expression studies. Insulin stimulated TNF-alpha secretion in a dose dependent manner (p<0.01) while the presence of RSG (10-8M) reduced TNF-alpha secretion (p<0.05).

Conclusions: Long-term insulin treatment of human adipocytes stimulates lipolytic rate, in a dose dependent manner. Co-treatment with RSG sensitises the effect of insulin to further alter LPL and HSL protein expression. These data, therefore, suggest an explanation for insulin resistance and hyperinsulinaemia paradoxically co-existing with increased circulating non-esterified fatty acids in obese and/or type 2 diabetic patients.

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ANTI-DIABETIC EFFICACY OF GW0072, A SELECTIVE PPAR_γ MODULATOR, IN ZUCKER DIABETIC FATTY RATS.

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Background and Aims: GW0072 is a high affinity Selective PPAR_γ Modulator (SPARM) that inhibits PPAR_γ-induced adipocyte differentiation as a result of its unique receptor-binding mode. The purpose of this study was to compare the anti-diabetic effects of the SPARM, GW0072, to that of the full PPAR_γ agonist, GW7845, at a dose providing comparable glucose (G) lowering. **Materials and Methods:** The Zucker diabetic fatty rat (ZDF) is a genetic model of Type 2 diabetes, becoming progressively insulin resistant, hyperglycemic and hyperlipidemic as they age. 40 male ZDF rats (9 wk of age) were dosed with either vehicle (PEG400), GW0072 (100 mg/kg bid) or GW7845 (0.3 or 1 mg/kg bid) for 21 d. **Results:** ZDF rats treated with GW7845 decreased non-fasted G at both doses. Rats treated with GW0072 lowered G to the level of the low dose of GW7845 (279 ± 62.5 v. 264 ± 64.3 mg/dl). GW0072 also produced effects on hemoglobinA1c (HbA1c), triglycerides (TG), free fatty acids, and insulin that were equivalent to the low dose of GW7845. Notably, only 4/8 animals treated with GW0072 or 5/8 treated with the low dose of GW7845 responded to treatment (G < 250 mg/dl), yet those that did responded maximally compared to the high dose of GW7845. Results from glucose-matched groups of all non-fasted ZDF rats are shown below (mean ± SEM).

Dose	G (mg/dl)	HbA1c (%)	TG(mg/dl)	Responders
Vehicle	472 ± 14.8	9.04 ± 0.15	893 ± 90	0/8
GW0072 100g/kg	279 ± 62.5	7.06 ± 0.67	624 ± 97	4/8
GW845 0.3mg/kg	264 ± 64.3	6.55 ± 0.68	420 ± 101	5/8
GW845 1mg/kg	135 ± 3.6	5.36 ± 0.25	114 ± 6.5	8/8

Analysis of gene expression in the responders showed that GW0072 did not upregulate lipid storage genes in fat (e.g. GPAT and FABP) to the same extent as GW7845, but showed equivalent regulation of genes in muscle involved with glucose disposal (e.g. PDK4). **Conclusions:** The SPARM, GW0072, showed anti-diabetic activity through tissue selective regulation of gene expression in a subset of ZDF rats and may be an effective therapy to improve glycemic control in patients with Type 2 diabetes.

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IMMEDIATE ONSET OF DIABETES IN ZDF RATS AFTER TERMINATION OF TREATMENT WITH ROSIGLITAZONE

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Background: It is well established that rosiglitazone, a thiazolidinedione, induces adipocyte differentiation by binding to and activation of PPAR_γ. The in-vivo effect of this drug is characterized by an increase in body weight, a fall in triglycerides and free fatty acids and increased insulin sensitivity. Male Zucker diabetic fatty (ZDF) rats are insulin resistant and develop diabetes at around 10 weeks of age presumably due to lipotoxic destruction of pancreatic β-cells. Aim of the study was to investigate the diabetes prevention properties of rosiglitazone and the effect of termination of treatment on diabetes development.

Material and Methods: Insulin resistant ZDF rats (fa/fa) were used (n=32). Rats were treated with rosiglitazone (3 mg/kg orally) starting at the age of 6 weeks (n=16). At the age of 19 weeks rosiglitazone treatment was terminated in 8 rats and the observation period was prolonged for further 6-weeks. A group of 16 rats without treatment served as control. Blood parameters and body weight were determined throughout the study.

Results: Treatment with rosiglitazone caused a fall in plasma triglycerides and free fatty acids whereas cholesterol and glycerol increased. Treatment prevented the onset of diabetes at the age of 10 weeks and caused a tremendous increase in body weight up to 682±11 g and 824±11 g at the age of 19 and 25 weeks, respectively. Corresponding body weights of control rats were 465±11 g and 459±18 g. After termination of treatment there was an immediate onset of diabetes within 1 week with increased triglycerides and free fatty acids and subsequent reduction in body weight.

Conclusions: It is concluded that termination of rosiglitazone treatment in the insulin resistant prediabetic disease stage might cause an immediate transition to overt diabetes.

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THIAZOLIDINEDIONES HAVE A DISTINCT INFLUENCE ON PLASMA STEROIDS IN OBESE ZUCKER RATS (fa/fa).

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Background and Aims: Thiazolidinediones (TZDs) have been reported to affect steroid synthesis *in vitro*. This study was to examine the potential influence of TZDs on circulating plasma steroids of obese Zucker rats *in vivo*. **Materials and Methods:** 8 months-old male genetically obese Zucker rats (fa/fa) received a food admixture of rosiglitazone (ROSI; 0.01% wt/wt; n=6) or troglitazone (TRO; 0.3% wt/wt; n=10) for 5 weeks, and were compared to untreated obese Zucker rats (fa/fa; n=12) and to untreated lean Zucker rats (Fa/-; n=12). **Results:** ROSI and TRO ameliorated the insulin resistance and improved the plasma lipid profile of obese rats (e.g. plasma triglycerides, mg/dl: ROSI, 213±40, vs TRO, 238±28, vs untreated obese, 751±107, vs untreated lean, 69±6; p<0.001 each vs untreated obese). Both TZDs markedly increased plasma 17-OH-progesterone (ng/dl: ROSI, 48±9, vs TRO, 46±5, vs untreated obese, 27±3, vs untreated lean, 58±10; p<0.05 each vs untreated obese), but failed to affect plasma pregnenolone (ng/dl: 18±2 vs 20±3 vs 19±1 vs 16±2; n.s.). While only a tendency to decrease plasma testosterone was observed (ng/dl: 123±35 vs 123±12 vs 162±29 vs 148±33; n.s.), both ROSI and TRO blunted plasma dihydrotestosterone by -50% (ng/dl: ROSI, 44±3, vs TRO, 38±4, vs untreated obese, 76±9, vs untreated lean, 59±7; treated vs untreated obese, p<0.01 each; untreated obese vs lean, n.s.). It is of note that TZDs reduced plasma dihydrotestosterone in obese rats beyond the concentrations prevailing in healthy rats, which contrasts with partial restoration of fuel metabolism and hence argues for different underlying mechanisms. **Conclusions:** ROSI and TRO have a similar and distinct influence on plasma steroids of obese Zucker rats. The precise mechanisms responsible for TZD-induced changes in the steroid profile of insulin resistant rats await to become clarified, but our data suggest a TZD group effect, which is not secondary to improved fuel metabolism. No conclusions can be drawn as yet regarding the extent, to which our data relate to circulating steroid hormones in TZD treated patients.

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Modifying effects of vitamin A in combination with thiazolidinedione treatment

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Background and Aims: We already reported thiazolidinedione treatment increases fat deposition in muscle and decreases fat deposition in liver accompanied with body weight gain. Vitamin A is reported to contribute to fat deposition in muscle and liver. We evaluated modifying effects of vitamin A in combination with thiazolidinedione treatment on fat deposition in wister rats with high-fat, high-sugar feeding (26% fat, 32% sugar) without food intake restriction. **Materials and Methods:** Thirty male wister rats were randomly divided into three groups and followed for 16 weeks. Group T (n=10) was treated with 0.2% troglitazone. Group TA (n=10) was treated with 0.2% troglitazone and vitamin A (approximately 500IU/body/day). Group C (n=10) was a control group. **Results:** Although there were no significant differences in food intake (g) between three groups after 16 weeks, body weight significantly (p<0.01) increased in group TA compared with those in the other groups. Body weight in group T also significantly (p<0.01) increased compared with group C. Triglyceride deposition in liver and psoas muscle in group TA (12±6 and 8±6µg/mg, respectively) were significantly (p<0.01) increased compared with those in group T (3±1 and 3±3µg/mg, respectively) and group C (3±1 and 1±1µg/mg, respectively). Although triglyceride deposition in psoas muscle also increased in group T compared with that in group C, it was not significantly different. Fasting IRI significantly decreased in group TA compared with those in group T and group C (group TA vs group T and group C: 2.3±0.7 vs 5.3±1.6 and 6.1±1.8µU/ml, p<0.01 and p<0.01, respectively). FPG significantly increased in group TA compared with those in group T and group C (175±21 vs 129±22 and 139±40mg/dl, p<0.01 and p<0.05, respectively). Although portal FFA significantly (p<0.01) decreased, serum triglyceride significantly (p<0.01) increased in group TA compared with those in the other groups. **Conclusions:** Vitamin A treatment enhanced the effects of thiazolidinedione to decrease hyperinsulinemia and FFA, it resulted in fat deposition spoiling hypoglycemic effect. This may be a key mechanism of a secondary failure of thiazolidinedione treatment. These results indicate vitamin A has modifying effects on insulin sensitizer.

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INSULIN SENSITIZATION BY PROLONGED TROGLITAZONE EXPOSURE OF RAT SKELETAL MUSCLE *IN VITRO*.

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Aims: It is unknown, whether thiazolidinediones trigger insulin sensitization of skeletal muscle directly or indirectly, i.e. via their actions on adipose tissue. We examined the direct effects of troglitazone on skeletal muscle *in vitro*. **Materials and Methods:** Freshly isolated soleus muscle strips from Sprague-Dawley rats were incubated with or without troglitazone. Since the induction of insulin sensitivity by troglitazone *in vivo* is a slow process, prolonged exposure periods of 73 h were employed. During the last hour, basal and insulin-stimulated (100 nmol/l) rates of net glycogen synthesis and glucose oxidation were determined. Glycogen content was measured at the end of the experiment. **Results:** Troglitazone exposure for 73 h dose-dependently increased insulin-stimulated glycogen synthesis and glucose oxidation of isolated muscle (% change vs. intraindividual troglitazone-free control: glycogen synthesis: 0.1µM, +14±12%, ns; 0.3µM, +1±11%, ns; 1µM, +49±13%, p<0.01; 3µM, +104±22%, p<0.01; glucose oxidation: 0.1µM, +19±14%, ns; 0.3µM, +14±15%, ns; 1µM, +57±11%, p<0.001; 3µM, +93±29%, p<0.01; glycogen content: 0.1µM, +10±8%, ns; 0.3µM, +10±11%, ns; 1µM, +33±8%, p<0.001; 3µM, +58±24%, ns). Another experiment revealed that troglitazone's effect on glycogen synthesis depended on the concomitant presence of insulin (% change induced by 1µM troglitazone: basal, -4±6%, vs. insulin-stimulated, +57±15%, p<0.01), while the troglitazone-induced increase in glucose oxidation was insulin-independent (basal, +62±22%, vs. insulin-stimulated, +56±18%, ns). **Conclusions:** Long-term troglitazone exposure of native muscle specimens leads to insulin sensitization of the glycolytic pathway accompanied by an insulin-independent increase in glucose oxidation. The observed changes exactly match the pattern of responses described for muscle specimens as prepared from rodents after oral thiazolidinedione-treatment. Our findings therefore suggest that troglitazone-induced insulin sensitization *in vivo* may at least in part be caused by direct interaction with skeletal muscle.

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Estimation of Insulin Resistance

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ACANTHOSIS NIGRICANS (AN) IN OBESE WOMEN OF AN AFRICAN AMERICAN ANCESTRY POPULATION. L.M.B. Araújo, A. M. C. Viveiros, R.C. Lopes, M. V. Porto, M. J. Ursich. Faculdade de Medicina da UFBA, Bahia, Brazil & USP, São Paulo.

AIMS: To study the prevalence of AN and metabolic disturbances in a population of obese women from Salvador, Bahia, Northeast of Brazil, a population predominantly of African ancestry. **CASUISTIC AND METHODS:** A total of 481 obese women of an outpatient obesity clinic was studied: with AN, group AN, $n=388$, and without AN, group NAN, $n=93$. The mean age of this population was 36.6 ± 10 years and mean BMI was 40.4 ± 6.4 kg/m². Except for those who were diabetic, all patient were referred to an oral glucose tolerance test (75g) and had plasma glucose determined by enzymatic method. Insulin resistance was evaluated by HOMA model. **RESULTS:** The prevalence of AN was 66.9% in patients with white skin color, 86% in mulattos and 90.6% in blacks, strongly higher in black and mulatto versus white ($p = 0.000001$ and 0.00002). The AN group was older, and heavier, they had a higher waist, waist-hip ratio (WHR) and HOMA insulin resistance than NAN group. In contrast, fasting insulin levels were similar between groups. The frequency of diastolic hypertension was higher in AN than NAN group ($p = 0.05$). The frequencies of central obesity (WHR > 0.85), type 2 diabetes and impaired glucose tolerance (according WHO criteria) in AN group were 38%, 11.1% and 14.9% higher than 29.7%, 4.3% and 9.7% in NAN group, but these differences were significant for diabetes only ($p<0.01$). The frequencies of high total and LDL-cholesterol, low HDL-cholesterol and high triglycerides of AN group were similar to NAN group. **CONCLUSION:** AN is a marker of some clusters of metabolic syndrome, especially in a multi racial population.

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SUCCESSFUL SCREENING FOR INSULIN RESISTANCE USING HOMA TOGETHER WITH A SIMPLE HEALTH EXAMINATION

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Background: Insulin resistance (IR) is a very important risk factor for many metabolic disorders. Therefore earlier detection and prompt educational intervention are desirable to reduce the prevalence of further metabolic diseases and their complications. **Aims:** We have assessed whether individuals at high risk of IR could be identified following a health examination and postprandial blood testing. **Materials and Methods:** Two hundred and eighteen asymptomatic, undiagnosed male workers (aged 21 -61) who were found to have glucosuria in an annual health examination were enrolled in this study. All underwent physical examination, 75gOGTT, and blood examination in fasting and postprandial state. Homeostasis model assessment (HOMA-IR) was calculated using the results of 75gOGTT. IR was defined as values above the 85th percentile. **Results:** The prevalence of IR in 20 - 29 age group was higher than in 50 - 59 age group (27.6% vs. 4.3%, $p = 0.0003$). Discriminant analysis was performed by the stepwise method using HOMA-IR as the dependent variable, and age, BMI, systolic blood pressure, diastolic blood pressure (D-BP), postprandial blood data as the independent variables. The coefficients were -0.053 for age, 0.254 for BMI, 0.027 for D-BP, and -5.934 for constant (Wilks' lambda 0.641, $p<0.0001$). The discriminant scores (IR risk scores) were computed using the coefficients. When subjects were discriminated by the score, the sensitivity and specificity in this study were 89.7%, and 78.8%. **Conclusions:** IR could be predicted with considerable accuracy from age, BMI, and D-BP, without the need for any blood data. This method should be useful to identify apparently healthy individuals with IR, especially younger individuals, after a simple health examination, facilitating early educational intervention to prevent many metabolic disorders.

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METABOLIC IMPROVEMENT IN OVERWEIGHT MEN FOLLOWING EXPOSITION TO MODERATE ALTITUDE

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Background and Aims: The metabolic syndrome is associated with an increase in sympathetic nervous activity, which is known to be modulated by a stay at different altitudes, but until now no data are available about the influence of altitude on parameters of the metabolic syndrome.

Methods: 22 male subjects with a metabolic syndrome were selected, and, after baseline investigations, these participants spent 3 weeks at 1700 m altitude simulating a holiday stay. Fasting glucose, glycated hemoglobin (HbA1c) and lipid values were determined according to routine procedures. As a measurement for insulin resistance Homeostasis Model Assessment (HOMA) was used. Statistic values are given as medians, for univariate testing the paired t-test was applied.

Results: Fasting plasma glucose levels decreased from 109.2 ± 33.6 mg/dL at the baseline control to 102.1 ± 61.8 mg/dL after the stay at moderate altitude, and HbA1c levels decreased from $6.3 \pm 1.5\%$ to $4.6 \pm 0.53\%$ ($p<0.069$). Fasting insulin levels were 16.2 ± 11.3 μ U/mL at the baseline control, and 11.6 ± 5.9 μ U/mL after the stay in the mountains, which was paralleled by a decrease in the HOMA index from 4.8 ± 4.1 to 3.3 ± 1.9 ($p<0.036$). Serum triglyceride levels decreased from 218.6 ± 135.5 mg/dL to 96.3 ± 116.9 mg/dL, while HDL-cholesterol revealed an increase from 45.5 ± 12.3 mg/dL to 54.7 ± 4.6 mg/dL ($p<0.001$).

Conclusion: These results indicate that a stay at moderate altitude might increase insulin sensitivity in overweight men.

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Validation of an insulin resistance score calculated from clinical parameters in a heterogeneous population with prior gestational diabetes.

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Background and Aims: Since exact estimation of insulin resistance requires invasive and expensive techniques, it would be important to find easily obtainable parameters to estimate this cardiovascular risk factor. Recently an insulin resistance score (IRS) was developed and validated in type 1 diabetes (T1DM) in the EDC Study. Our aim was to calculate this parameter and validate it using different measures of insulin resistance from an oGTT (homeostatic model assessment [HOMA], Matsuda composite index, Cederholm, and Stumvoll estimation) in a heterogeneous population. **Materials and Methods:** We examined 84 prior GDM women (age $38.6 [6.3; SD]$ yrs, HbA1c $6.4 [1.9]$ %, BMI $26.8 [6.1]$ kg/m², 22.4% hypertensive) 7.7 [2.4] yrs after delivery. Almost half of the patients had normal glucose tolerance, 18% had impaired glucose tolerance and 31% diabetes at reclassification (WHO criteria). HOMA score was calculated on all subjects using fasting serum insulin and fasting plasma glucose, while the other measures of insulin resistance were calculated using blood glucose and serum insulin levels measured on a mixed subgroup of the patients ($n=43$) during a 75g oGTT. To estimate IRS, each patient was categorized into risk tertiles according to waist to hip ratio (WHR), triglycerides, HDL-cholesterol, and HbA1c levels. Hypertension (HTN) and known diabetes in first-degree relatives was considered as a high risk, their absence as a low risk factor. These scores were then averaged creating a score between 1 (low risk) and 3 (high risk). **Results:** IRS correlated well with all the insulin resistance measures: with HOMA scores, composite index, Cederholm and Stumvoll estimation ($r=0.57, 0.44, 0.39, 0.33, P<0.0001, P<0.01, P<0.05, P<0.05$, resp.). It even correlated well with HOMA score in different subgroups with no substantial difference in younger ($r=0.37, P<0.05$) or older ($r=0.76, P<0.001$), obese ($r=0.41, P<0.05$) or non-obese ($r=0.47, P<0.001$), diabetic ($r=0.43, P<0.001$) or non-diabetic ($r=0.46, P<0.05$), and in normotensive ($r=0.42, P<0.001$) or hypertensive subjects ($r=0.48, P<0.05$). ROC curve analysis also revealed that IRS score could also well differentiate between diabetic and non-diabetic women (AUC= 0.82, 95% CI: 0.72-0.91, $P<0.0001$) with a sensitivity of 88% and a specificity of 66% at a cut-off value of 1.77. **Conclusions:** Our results might support the previous observation that easily measurable clinical parameters may estimate insulin resistance. The calculated score could help in large-scale epidemiological studies even in heterogeneous populations.

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Assessment of insulin sensitivity in children and adolescents. Comparison of quantitative approaches.

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Background and Aims. We have compared different methods of calculating insulin sensitivity from glucose and insulin basal values and from an oGTT in order to have an alternative approach of euglycemic hyperinsulinemic clamp for routine measurement in population studies.

Materials and Methods. b-cell function was obtained from fasting glucose and insulin, Homa index (B%) and from an oGTT, the insulinogenic index (insulin area divided by glucose area) and MSI (mean serum insulin, the area under the insulin curve divided by 120 minutes). Insulin sensitivity was assessed by the homeostasis model assessment (Homa S%), a well validated method, which has been compared with quantitative insulin sensitivity index (QUICKI), fasting insulin resistance index (FIRI), glucose and insulin ratio (G/I), fasting glucose and insulin ratio (FGIR) and insulin sensitivity index calculated from basal and oGTT (ISIb and ISla).

The population included were 51 girls diagnosed of premature pubarche, studied longitudinally at prepubertal, pubertal and postpubertal stages and 68 matched controls for age, sex and pubertal stages.

Results. High correlation coefficients (ranging from 0.87 to 0.92) were found between Homa S% and the other insulin sensitivity indexes adjusted either linearly or as a hyperbolic curve. Assuming that the relationship between insulin secretion and insulin sensitivity is represented by a hyperbolic function, we found three acceptable adjustments, between B% and G/I, MSI and ISla and the insulinogenic index with ISla. The best adjustment fitting a hyperbolic relation was considered to be between MSI and ISla ($r^2 = 0.90$).

Conclusions. According to our results it may be considered that S%, G/I and ISla the most advantageous calculations to represent a simple value of insulin sensitivity in a population from which a subset of them could be submitted to a more accurate an sophisticated method.

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PROTEASE INHIBITORS CAUSE INSULIN RESISTANCE AND IMPAIR BETA CELL FUNCTION: A MODEL TO STUDY THE PATHOGENESIS OF TYPE 2 DIABETES

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Background: There is evidence that Protease inhibitors (PI), drugs commonly used for HIV infection, may be diabetogenic. However, no prospective study has examined this. Furthermore it is not clear what effects, if any, PI treatment may have on insulin release (IR) and insulin sensitivity (IS). We therefore undertook a 12 week prospective study to determine whether PI treatment impairs glucose tolerance as reflected by changes in fasting plasma glucose (FPG) and, if so, the mechanism(s). **Methods:** We measured FPG, beta cell function (BCF) and IS before and after 12 weeks PI treatment, using both the HOMA model and the hyperglycemic (180 mg/dl) clamp technique in 10 HIV positive otherwise healthy subjects. With the clamp technique, IR was calculated as the average plasma insulin concentration during the last 60 min of the 3 hour clamp. IS was calculated by dividing glucose clearance during the last 60 min of the clamp by the corresponding plasma insulin concentrations. **Results:** FPG increased from 89 ± 4 to 98 ± 2 mg/dl $p=0.009$. Fasting plasma insulin increased from 92 ± 14 to 108 ± 12 pM, $p=0.028$. The HOMA model indicated that IS decreased from 0.6 ± 0.1 to 0.3 ± 0.1 $\text{lpmol}^{-1}\text{mol}^{-1}$ $p=0.004$ whereas BCF remained unchanged (204 ± 13 vs. 191 ± 15 pMmM^{-1} , $p=0.52$), indicating lack of appropriate BCF compensation. With the hyperglycemic clamp: IS decreased from 11.9 ± 2.2 to 8.9 ± 2.7 $\text{ml kg}^{-1}\text{min}^{-1}\text{nM}^{-1}$, $p=0.006$. Although IR increased from 642 ± 104 to 1101 ± 370 pM, $p=0.02$ this was not appropriate for the decrease in IS since the disposition index (ISxIR) decreased from 4913 ± 192 to 3963 ± 318 , $p=0.01$. There was a significant correlation between the decrease in insulin sensitivity determined with the HOMA and the hyperglycemic clamps $r=0.74$, $p=0.0004$ as well as for changes in BCF $r=0.69$ $p=0.029$. **Conclusions:** Protease inhibitor treatment reduces glucose tolerance. Both HOMA modeling and hyperglycemic clamp experiments indicate these drugs reduce insulin sensitivity and prevent appropriate compensation in beta cell function. Since these changes mimic those involved in obesity and Type 2 diabetes mellitus, these drugs may be useful tools to study the pathogenesis of T2DM.

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INSULIN SECRETION AND INSULIN RESISTANCE IN IMPAIRED GLUCOSE TOLERANCE

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Background and Aims: The study was designed to investigate insulin secretion and insulin resistance in normal-weight subjects with impaired glucose tolerance (IGT).

Materials and Methods: 114 normal-weight subjects (64 males and 50 females) were enrolled in the study - 39 subjects with IGT (mean age 39.2 ± 8.3 years); 41 newly-diagnosed type 2 diabetic patients (mean age 38.5 ± 7.9 years) and 34 healthy controls (mean age 37.4 ± 9.1 years). First (FPIS) and second (SPIS) phases of insulin secretion were studied during IVGTT. Insulin resistance was estimated using the homeostasis model assessment (HOMA-IR). Plasma insulin concentration was measured by MEIA, using a commercial kit (Abbott) (normal range - 2-25 mU/l). Statistical analysis was performed with a SPSS 9.01 package. **Results:** As far as their response during IVGTT is concerned, the subjects with IGT were divided into two subgroups: with normal FPIS ($n=22$, 108.4 ± 27.9 mU/l); and with reduced FPIS ($n=17$, 26.7 ± 8.2 mU/l vs 114.4 ± 1.2 mU/l in the controls, $p<0.001$, and $p<0.05$ as compared to the diabetic patients) and reduced AUC for insulin secretion (1611.7 ± 481.9 vs 2318.8 ± 734.9 mU/l.h in the control group, $p<0.001$), the pattern of insulin secretion being quite similar to that of type 2 diabetic patients. The SPIS of the two IGT groups did not differ from that of the healthy controls. 18 of the subjects with IGT were with normal insulin sensitivity (HOMA-IR) (2.43 ± 0.89 vs 2.08 ± 1.4 mmol/l.mU/l in the control group, $p>0.1$); 21 of them showed insulin resistance (4.13 ± 1.4 , $p<0.01$ as compared to the control group), but they were less resistant than the newly-diagnosed type 2 diabetic patients ($p<0.05$). Having combined the two defects, 23.1% of IGT subjects appeared to have both reduced FPIS and insulin resistance; 20.5% - only reduced FPIS; 30.8% - just insulin resistance and 25.6% - both normal insulin secretion and insulin sensitivity. **Conclusions:** Our results demonstrate that both insulin secretion and insulin sensitivity defects may be present in normal-weight subjects with IGT.

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Glucose Metabolism

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ABDOMINAL SAGITTAL DIAMETER IS STRONGLY RELATED TO INSULIN RESISTANCE IN MEN WITH THE METABOLIC SYNDROME
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Background and Aims: Abdominal sagittal diameter (SAD), an estimate of visceral fat, is strongly related to cardiovascular and metabolic risk factors, but its association with insulin resistance (IR) has not been examined using clamp. Here we compared simple anthropometric measures and their association with IR.

Materials and Methods: In a homogeneous sample of 59 men with the metabolic syndrome (mean±SD; 53±8.2 yrs, BMI; 30.6±2.3, waist to hip ratio; 1.01±0.02) euglycaemic hyperinsulinemic clamp (M; mg/kg bw/min) were assessed, and correlations between anthropometrics and the M-value were analysed.

Results:

Anthropometric variable	Correlation coefficient, r	P-value
Abdominal sagittal diameter	-0.57	P<0.0001
Body mass index	-0.46	P=0.002
Waist circumference	-0.41	P=0.001
Waist to hip ratio	-0.41	P=0.003

In addition, SAD was the strongest correlate for fasting insulin, glucose and M corrected for insulin levels (M/I).

Conclusions: Despite the homogenous group, SAD was the best correlate of IR, which probably reflects the important role of visceral fat in IR, but also the high measurement reliability of SAD compared to other anthropometrics. These results indicate that among prediabetic men, SAD identifies the most insulin resistant individuals and may have higher predictive value for NIDDM compared to BMI, waist circumference and waist to hip ratio.

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INSULIN RESISTANCE IS ASSOCIATED WITH PLATELET RESISTANCE TO ACETYSALICYLIC ACID IN VIVO IN OBESITY
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Background and Aims: Resistance to insulin's antiaggregatory effects *in vitro* characterize platelets of obese subjects but it is unknown whether obesity also influences the sensitivity of platelets to acetylsalicylic acid (ASA) *in vivo*. **Materials and methods:** We determined insulin sensitivity of glucose uptake by using the euglycemic insulin clamp technique, and ASA sensitivity of platelets by measuring aggregation responses to 4 doses (0.5, 0.75, 1 and 1.5 mmol/l) of arachidonic acid (AA) before and 1 hour after ingestion of 50 mg ASA in eight non-obese (age 49±3 yrs, BMI 25±1 kg/m²) and seven obese subjects (age 50±3 yrs, BMI 32±1 kg/m²). *In vitro* platelet aggregation was measured with an aggregometer using platelet rich plasma and Born's turbidometric method. The average of the percent changes in maximal aggregation recorded at the four doses of AA was calculated (ASA sensitivity). **Results:** Whole body glucose uptake was 48 % lower in the obese (3.9±0.8) than the non-obese (7.5±0.6 mg/kg-min, p<0.01) subjects. AA induced maximal aggregation in all subjects before ASA (93±1 % in non-obese and 94±1 % in obese, NS). After 50 mg oral ASA, maximal aggregation decreased to 19±3 % in the non-obese but only to 79±6 % in the obese subjects (p<0.0001). There was significant correlation between body mass index and inhibition of aggregation by ASA (r=0.72, p=0.0024). ASA sensitivity was also significantly correlated with insulin sensitivity (r=-0.61, p=0.0148). **Conclusions:** These data demonstrate that insulin and ASA sensitivity are interrelated *in vivo* in non-diabetic individuals. ASA resistance could contribute to the increased risk of atherothrombosis in insulin resistant individuals. Insulin resistant obese subjects need a greater ASA dose than normal-weight subjects.

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URINARY ACTIVITY OF N-ACETYL BETA-GLUCOSAMINIDASE (NAG) IS AN INDICATOR OF SHORT AND LONG-TERM METABOLIC CONTROL

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Increased secretion of lysosomal enzymes into extracellular fluids does not have to be only the result of cellular disintegration, but may occur due to alterations in intracellular transport and release. The aim of the study was to examine significance of urinary and serum NAG activity as the parameter of metabolic control. The study included 70 patients: 30 Diabetes mellitus type 1 (DM 1) newly diagnosed and patients without complications, 20 Diabetes Mellitus type 2 (DM 2) patients without complications, unsatisfactory metabolic control and 20 healthy control patients. NAG in the serum and urine has been determined spectrophotometrically with substrate glucopiranozide. Glucosylated haemoglobin (HbA1C) by spectrophotometric method and glucosylated proteins (GP) by thiobarbituric acid have been determined as glycosylation parameters. **RESULTS:** Correlation of NAG serum and urine activity with parameters of long term metabolic control (HbA1C and GP) in the DM 2 patients without vascular complications has shown correlation of NAG serum activity with HbA1C r = 0.82, p<0.05 and GP r=0.84, p<0.05, and correlations of NAG urine activity with HbA1C r = 0.98, p<0.05 and GP r = 0.98 p< 0.05. Correlation of NAG urine activity with parameters of short-term metabolic control (basal glycaemia) in the newly diagnosed DM 1 patients and patients without vascular complications r = 0.79, p< 0.05. These results confirm that NAG urine activity is an indicator of short and long-term metabolic control, and serum NAG is an indicator of long-term metabolic control in patients without vascular complications. The correlations between elevated urinary NAG and hyperglycaemia may indicate that the tubular cells are sensitive to plasma glucose or increased tubular glucose reabsorptions.

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EVALUATION OF GLUCOSE METABOLISM IN TYPE 2 DIABETES MELLITUS

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Background and Aims: Glucose metabolism in the body determines in many respects the energy metabolism level. The aim of the study was to investigate the rate of glucose oxidation and to evaluate the pyruvate dehydrogenase complex state in patients with type 2 diabetes mellitus (DM) by stable isotope methodology. **Materials and Methods:** Glucose oxidation rate was studied based on measurement of ¹³C isotope concentration in expired air by the mass spectrometry method (¹³CO₂) and simultaneous measurement of lactate and pyruvate levels in 7 patients with type 2 DM and 4 apparently healthy subjects aged 35 to 55 years.

The type 2 diabetic patients had compensated carbohydrate metabolism (HbA_{1c} = 6.8 ± 0.59%). All the subjects studied received 20 mg, 10g and 20g of ¹³C glucose per os at different days at an interval of 2-3 days. **Results:** See the Table below.

¹³ C gluc. dose	Rate of glucose oxidation [1x10 ⁻³]		Lactate [mmol/l]		Pyruvate [μmol/l]	
	Healthy	DM	Healthy	DM	Healthy	DM
20 mg	8.3±0.25	4.3±0.17*	1.2±0.25	1.9±0.2*	57.2±5.3	6.1±7.1
10g	9.5±0.33	5.1±0.67*	1.3±0.11	1.8±0.3*	65.1±6.6	56.9±9.2
20g	11.9±0.51	3.95±0.57*	1.6±0.21	2.5±0.4*	78.1±7.2	54.3±8.1

p* < 0.05

Conclusions: Glucose oxidation rate in healthy subjects increases with glucose test load and they have increased pyruvate level without hypoxia shift. Patients with type 2 DM show decreased rate of glucose oxidation as glucose load increases with sharp increase of tissue hypoxia evidence and signs of pyruvate dehydrogenase complex fermentative systems depletion (increase of lactate and decrease of pyruvate level in blood), that demonstrates an important role of this complex in carbohydrate metabolism regulation processes.

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METABOLIC CONTROL AND SPLANCHNIC GLUCOSE UPTAKE, INSULIN SENSITIVITY AND GLUCOSE ABSORPTION TIME IN TYPE-1 DIABETES
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Background and Aims: Insulin sensitivity and splanchnic glucose uptake (SGU) are important regulators of postprandial glycaemia. While SGU is decreased in type-2 diabetes, existing data on the extent of SGU following oral glucose administration in type-1 diabetic patients are controversial, which might be caused by the different glycaemic control in the subjects investigated. The aim of the following study was to determine SGU, peripheral glucose uptake and the time required for glucose absorption in type-1 diabetic patients in relation to metabolic control using the OG-Clamp method.

Material and Methods: An OG-Clamp was performed in 8 type-1 diabetic patients with good metabolic control (DG, HbA1c=6.1±0.5 %, BMI=23.9±1.1 kg/m²), in 8 type-1 diabetic patients with poor metabolic control (DP, HbA1c=8.5±0.5%, BMI=25.5±1.5 kg/m²) and in 9 healthy, matched control subjects (C). The non-invasive OG-Clamp, which has been validated against conventional methods, combines a hyperinsulinemic clamp (120 mU/m²/min) with an oral glucose load (75g) during steady state of glucose uptake and yields SGU, peripheral glucose uptake and the time required for glucose absorption in one experiment.

Results: SGU was not different between the respective groups (13.7±6.2 % in C, 17.8±3% in DG and 18.9±4 % in DP, p=ns). Peripheral glucose uptake was slightly decreased in DP compared to C (7.6±1 vs. 9.3±0.7 mg/kg/min, p=0.08). The time required for glucose absorption was 140±6 min in C, 156±4 min in DG and 143±7 min in DP (p=ns).

Conclusions: In contrast to type-2 diabetic patients, SGU and the time required for glucose absorption is not different from healthy controls in patients with type-1 diabetes regardless of glycaemic control. In the patients with poor metabolic control, peripheral glucose uptake was slightly, but not significant decreased.

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Thermic effect of glucose in diabetes mellitus

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Background: Thermic effect of food is the amount of energy, which is necessary for its oxidation. In a mixed meal it is calculated as 10% of the contents of energy and this ratio is similar in parenteral and enteral nutrition. The values of thermic effect of nutrients are approximately 5% for glucose, 24% for protein, and 15 % for lipids.

Aim of study: To compare the thermic effects of glucose in diabetic patients and normal volunteers.

Patients and methods: 26 diabetes mellitus type 2 patients (age 54.5±8.5 years, BMI 29.7±5, fasting blood glucose 7.5±2.6 mmol/l, fasting insulinaemia 24.9±20.1 mU/l, HbA1c 7.9±1.5 %) and 12 healthy controls were enrolled into the study. A 3-hour oral glucose tolerance test (75 g glucose) with frequent blood sampling (insulin, glucose) associated with indirect calorimetry measurement for 20 minutes in a canopy mode (Deltatrac, Datex) was performed in the morning hours in a fasting state. The variables were tested at the times 60, 120, 180 min. after the ingestion of glucose. Insulin resistance severity was calculated from the insulin and glucose blood levels (Matsuda, 1999) and it was correlated to energy expenditure (EE/m²), CO₂ production and respiratory quotient (RQ).

Statistical analysis: Means ± SD were calculated for the group specification and the non - paired Wilcoxon test and analysis of variance (ANOVA) were used for the statistical analysis.

Results: In the basal state, the EE/m², CO₂ and RQ values were comparable between the groups. In diabetic patients, the EE/m² showed no significant differences in time (953±95.6, 964±101.4, 944±86.0, 935±78.1). In volunteers, EE/m² increased at 60 min. (909±166.3, 985±174.9; p<0.001), it decreased at 120 min. (951±156.0; p<0.05) and it dropped to the basal value at 180 min. (908±129.9; p<0.001). The CO₂ production and RQ values had similar characteristics. The correlation between the parameters of insulin resistance was not significant.

Conclusion: The EE/m², CO₂ production and RQ were comparable in diabetic 2 patients and volunteers in a fasting state. The EE/m², CO₂ and RQ in healthy people increased significantly after ingestion of glucose with normalisation after 3 hours. The thermic effect of glucose was very low in diabetic patients. Nevertheless, this difference could not be explained with insulin resistance in this study.

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In vivo imaging of insulin receptors by positron emission tomography: pre-clinical evaluation of [124I]-iodine and of [124I]-iodine-labelled human insulin
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Background and Aims: To date, only indirect measures have been obtained to characterise insulin receptor binding properties in vivo in humans. We carried out ex vivo and in vivo animal studies to investigate whether positron emission tomography (PET) could be a suitable technique for the assessment of insulin receptor distribution and insulin binding in vivo.

Materials and Methods: Human insulin was labelled with [125I]-I sodium iodide or cyclotron-produced [124I]-I sodium iodide at the A14 tyrosine residue, and injected (1.1 – 1.4 MBq . kg⁻¹) into adult male Sprague Dawley rats. Animals were sacrificed at different times after the injection. Arterial blood and tissue samples were collected to determine radioactivity for information on biodistribution, time course, and metabolism. Pre-dosing with unlabelled insulin (10 IU i.v.) was exploited to determine the magnitude of specific receptor binding of insulin tracers in each tissue. In vivo studies were conducted using an ECAT EXACT HR++ scanner. A transmission scan was followed by a 60-min emission scan after injecting the animals with [124I]-I-labelled insulin (1.5 – 3.0 MBq).

Results: Distribution and clearance of the two tracers were comparable. Plasma clearance of radioactivity was fast. Uptake of radioactivity in myocardium, liver, pancreas, and gut was reduced by pre-dosing. Maximal specific uptake was achieved 10 min after injection in the myocardium and much earlier in the liver. Uptake in the brain was low but was halved by pre-dosing. Radioactivity in the kidney was increased after pre-dosing. PET imaging showed diffuse distribution of the tracer and allowed clear identification of myocardium, liver and kidneys.

Conclusions: In the rat, plasma insulin crosses the blood-brain barrier to bind to specific receptors in multiple brain areas. [124I]-insulin is a potential radioligand for in vivo quantification of plasma insulin binding to tissue receptors in humans.

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PARTITIONING GLUCOSE TRANSPORT/DISTRIBUTION AND DISPOSAL DURING EUGLYCAEMIC CLAMP

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Background and Aims: We have separated the effect of insulin on glucose transport/distribution (GT) and glucose disposal (GD) during glucose clamp using a dual tracer dilution methodology.

Materials and Methods: 6 healthy male subjects (age 33±3 yr, BMI 22.7±0.6 kg m⁻²; mean±SE) underwent a 4h two-stage (subjects 1 & 2: 1 and 8mU kg⁻¹ min⁻¹, subjects 3-6: 0.5 and 2.5mU kg⁻¹ min⁻¹; insulin infusion) double-tracer (background infusion of and cold glucose spiked with D-[6,6-²H₂]glucose and 3-O-methyl-D-glucose) euglycaemic clamp preceded by a 2h basal period (both tracers administered) and followed by a 2h recovery period. Overall 98 samples were taken every 2-10min and were analysed for insulin, glucose, D-[6,6-²H₂]glucose, and 3-O-methyl-D-glucose. A new two-compartment model described kinetics of the two glucose tracers and represented the effect of insulin on GT and GD. **Results:** Modelling 3-O-methyl-D-glucose showed that insulin stimulation of GT is saturable. GD could be represented as a non-saturable process. The model gave dose response curve of glucose clearance (V_{max} ~20ml kg⁻¹ min⁻¹, K_m ~70mU/l) and partitioned insulin effect into two components representing insulin sensitivity of GT (S_{GT}) and insulin sensitivity of GD (S_{GD}). At basal insulin, S_{GT} was higher than S_{GD} (39.3±5.6 vs 11.3±2.7×10⁻² ml kg⁻¹ min⁻¹ per mU/l at 10mU/l). With increasing insulin, S_{GT} decreased faster than S_{GD} with a point of intersection at ~70mU/l (S_{GT} and S_{GD} 5.2±1.1×10⁻² ml kg⁻¹ min⁻¹ per mU/l). At higher insulin S_{GT} was smaller than S_{GD} (1.0±0.3 vs 1.5±0.4×10⁻² ml kg⁻¹ min⁻¹ per mU/l at 200mU/l). **Conclusions:** In healthy subjects, saturability of glucose clearance at euglycaemia can be fully explained by saturability of GT. At plasma insulin below 70mU/l, insulin acts primarily by stimulating GT, at higher insulin by stimulating GD.

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Long-Acting Insulin Analogues

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SUPPRESSION OF SPLANCHNIC GLUCOSE PRODUCTION BY INSULIN DETEMIR IN HUMANS REFLECTS CHANGE IN NEFA AVAILABILITY.

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Background and Aims: Between-meal blood glucose control is due in part to control of glucose production by basal insulin. Fatty acid acylated insulins have been developed for reproducible basal insulin replacement in patients with diabetes mellitus. A soluble insulin analog, Lys³²⁹-(N^{tridecanoil}-tetradecanoyl) des(B30) human insulin (or insulin detemir, ID), binds to albumin resulting in protracted action. We examined splanchnic glucose balance (SGB) and non-esterified fatty acid (NEFA) extraction during peripheral ID or human insulin (HI) infusion. **Materials and Methods:** Paired experiments of ID or HI infusion at 10.8 pmol/min/kg for 540min (n=10 normal healthy males, age 29-48 years) were performed. Splanchnic A-V differences and blood flow were measured via femoral artery and hepatic vein sampling. D-glucose-6,6-d2 was infused throughout for glucose turnover assessment. Euglycemia (93±1 mg/dl) was maintained by labeled glucose infusion. **Results:** During ID infusion, splanchnic NEFA extraction decreased from basal of 0.10±0.02 to steady state of 0.03±0.01 mmol/min (p<0.01); and during HI from 0.09±0.02 to 0.001±0.002 mmol/min (p<0.01 vs. basal and ID). Consistent with the protracted action, ID dynamics (halflives) for suppression of NEFA extraction were slower vs. HI (57±10 vs. 21±3 min; p<0.01). Tracer-estimated whole body glucose production (EGP) was equally suppressed to 26±5% of basal for ID and HI. Concomitant SGB declined during ID from basal of 1.4±0.1 (production) to -0.3±0.2 mg/min/kg (uptake) at steady state; and during HI from 1.6±0.1 to -0.8±0.2 mg/min/kg (p=NS between groups). Dynamics of EGP and SGB suppression were similar to dynamics of NEFA extraction, albeit protracted for ID vs. HI (43±5 min vs. 23±2 min; p<0.01). A change in SGB was closely associated with a change in splanchnic NEFA extraction (r²=0.79 for ID, r²=0.90 for HI; p<0.05 for both). **Conclusions:** These data show 1) protracted action of ID suppression of glucose production and NEFA extraction, and 2) for both ID and HI, a decrease in splanchnic NEFA extraction is associated with a decrease in splanchnic glucose production. In humans, control of splanchnic glucose production by HI and ID appears closely linked to NEFA availability.

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EFFICACY AND SAFETY OF 6-MONTH TREATMENT WITH INSULIN DETEMIR IN TYPE 1 DIABETIC PATIENTS ON A BASAL-BOLUS REGIMEN

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Background and Aims: Insulin detemir is a soluble, basal insulin analogue with neutral pH. The efficacy and safety of insulin detemir was compared to NPH in a 6-month, multi-centre, multi-national, open, randomised, parallel trial in Type 1 diabetic patients on a basal (twice daily)-bolus regimen with human soluble insulin as bolus insulin.

Materials and Methods: A total of 460 patients with no clinically significant diseases or diabetic complications were exposed (287 men and 173 women).

Results: Mean (SD) age: 39.2 (12.9) years; mean duration of diabetes: 14.7 (10.0) years; mean BMI: 25.3 (3.3) kg/m²; mean HbA1c: 7.6 (1.2) % 421 (91.5%) patients completed the trial (insulin detemir: 212, NPH: 209). Insulin detemir provided similar glycaemic control when compared to NPH, as measured by HbA1c, 9-point blood glucose profiles and fasting plasma glucose (FPG) after 6 months of treatment. HbA1c was comparable for insulin detemir and NPH with an absolute mean difference between treatments (insulin detemir-NPH) of 0.08% point and a 95% CI of [-0.05; 0.22]. No statistically significant difference in FPG was found between treatments (p=0.78), and 9-point blood glucose profiles for insulin detemir and NPH were comparable. There was a tendency towards lower intra-patient variation in fasting blood glucose (FBG) for insulin detemir indicating more predictable FBG levels (not statistically significant, p=0.06). The safety profiles were comparable between the two treatments, with no safety concerns. The number of patients with hypoglycaemic episodes was not statistically significantly different between the two treatments.

Conclusions: Insulin detemir provided comparable glycaemic control and a similar safety profile when compared to NPH in patients with Type 1 diabetes on a basal-bolus regimen.

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DOSE RELATIONSHIP BETWEEN INSULIN DETEMIR AND NPH: A MULTI-CENTRE, OPEN, TWO-PERIOD TRIAL IN TYPE 2 DIABETIC PATIENTS

O. Schmitz¹, R. Gray², A. Kristensen³, A. Qvist³ and M. Axelsen³. ¹Aarhus University Hospital, Denmark; ²St. John's Hospital, United Kingdom; ³Novo Nordisk, Denmark. **Background and Aims:** Insulin detemir is a new soluble, basal insulin analogue with neutral pH developed to cover basal insulin requirements. Previous clinical trials have suggested that the molar dose of insulin detemir should be higher than that of NPH to obtain the same blood glucose (BG) control. The primary objective of this trial was to estimate the ratio between doses of insulin detemir and NPH required to give comparable mean BG profiles. **Materials and Methods:** 58 Type 2 diabetic patients (30 on NPH and 28 on human pre-mixed insulin (Pre-Mix)) were enrolled in the trial, which comprised two treatment periods: Period 1 (2 weeks) on NPH or Pre-Mix once or twice daily (patient's usual regimen) and Period 2 (up to 5 weeks) where patients on NPH were transferred to insulin detemir and patients on Pre-Mix were transferred to insulin detemir + human soluble insulin. The regimen remained unchanged throughout the trial. The dose of insulin detemir was adjusted to provide a mean BG profile equal to that obtained with NPH or Pre-Mix (± 10%). The following endpoints were analysed: The doses and the ratio between doses of insulin detemir and NPH required to obtain comparable BG profiles, the mean BG level following insulin detemir and NPH treatment, the incidence of hypoglycaemia and adverse events, and standard safety parameters. **Results:** Comparable mean BG profiles were obtained with insulin detemir and NPH in both treatment groups. The mean BG level was approx. 9.4 mmol/L at the end of each treatment period. The absolute mean difference (insulin detemir-NPH) in BG level at the end of each treatment period was 0.02 mmol/L, 95% CI [-0.22; 0.25] for the NPH group and 0.08 mmol/L, 95% CI [-0.15; 0.31] for the Pre-Mix group. The mean ratio between molar doses of insulin detemir and NPH was 4.06, 95% CI [3.23; 5.11] for the NPH group and 3.60, 95% CI [3.19; 4.07] for the Pre-Mix group. **Conclusions:** Comparable mean BG profiles could be obtained, when approx. 4 times higher molar doses of insulin detemir were used. Standard safety parameters and occurrence of hypoglycaemia and adverse events were similar for both treatment groups.

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LESS SYMPTOMATIC HYPOGLYCEMIA WITH INSULIN GLARGINE COMPARED TO NPH IN PATIENTS WITH TYPE 2 DIABETES.

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Background and Aims: Insulin glargine (Lantus®), a human insulin analog that provides constant peakless insulin absorption from the injection site, has a prolonged duration of action compared to NPH human insulin.

Materials and Methods: In a multicenter, randomized, parallel group study, insulin glargine was compared with NPH in patients with type 2 diabetes previously treated with once a day NPH. Patients received a once a day dose (at bedtime) of either insulin glargine or NPH and were allowed to use preprandial regular insulin as part of their daily regimen. A total of 100 patients (mean age 57.9 years, mean glycohemoglobin (GHb) 8.4%, mean fasting blood glucose [FBG] 9.3 mmol/L) were treated for up to 28 weeks.

Results: Patients treated with insulin glargine and NPH achieved similar reductions from baseline for GHb (-0.35% vs. -0.44%, respectively) and for FBG (-0.95 mmol/L vs. -1.13 mmol/L, respectively). The percentage of patients reaching target FBG of <6.66 mmol/L at endpoint was also similar for patients treated with insulin glargine (29.5%) compared to patients treated with NPH (22.7%; p=0.613). In addition, there was no difference between insulin glargine and NPH in the proportion of patients who achieved a GHb of either <7% or <8%. The baseline and endpoint mean total daily dose of insulin glargine (27IU, 33IU, respectively) was similar to NPH (26IU, 30IU, respectively). However, fewer patients reported at least 1 episode of hypoglycemia symptoms with insulin glargine (46.2%) than those treated with NPH (60.4%; p=0.0488). Furthermore, the percentage of patients reporting a symptomatic hypoglycemia event confirmed by a blood glucose value of <2.8 mmol/L was less with insulin glargine (17.3%) than with NPH (31.3%; p=0.0017). A similar percent of patients treated with insulin glargine (15.4%) reported nocturnal hypoglycemia symptoms compared to NPH (27.1%; p=0.0805).

Conclusions: Bedtime insulin glargine was as effective as bedtime NPH in improving glycemic control, but with fewer patients reporting hypoglycemia symptoms and confirmed hypoglycemia events.

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A bedtime carbohydrate snack for the prevention of nocturnal hypoglycaemia in Type 1-diabetic patients treated with once daily insulin glargine

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Background and Aims: The typical insulin concentration peak of NPH-insulin 3-4 hours after injection led to the recommendation of a bedtime snack in order to avoid nocturnal hypoglycaemia. The profile of insulin glargine is a constant insulin concentration over 24 hours. It was the aim to study, whether a bedtime snack has also to be recommended when using the long acting analogue.

Materials and Methods: 20 patients with Type 1 diabetes (12 female, 8 male; age 47 ± 14 years, diabetes duration 17 ± 13 years, BMI 26.6 ± 3.5 kg/m²) were studied twice (crossover-design). In a randomised protocol they received a bedtime snack (20g carbohydrate as coarse wholemeal bread) or not. From 6:00 p.m. to 8:00 p.m. of the following day blood glucose was frequently measured and between 10:00 p.m. to 8:00 p.m. of the following day symptomatic and chemical (< 2.8 mmol/l) hypoglycaemic episodes were recorded. Statistics: RM-ANOVA and Fisher's exact test.

Results: The plasma-glucose profile with and without bedtime snack was significantly different ($p < 0.0001$). The differences (lower values without bedtime snack) were evident at 0:00 a.m. (5.94 ± 0.56 mmol/l vs. 8.39 ± 0.5 mmol/l; 5.17 ± 0.56 mmol/l vs. 7.83 ± 0.61 mmol/l; both $p = 0.003$). While only one case of symptomatic hypoglycaemia was recorded with a bedtime snack, 9 patients had symptomatic hypoglycaemia (2.33 ± 0.11 mmol/l) and one has non-symptomatic hypoglycaemia (2.78 mmol/l; $p = 0.008$).

Conclusions: Nocturnal blood glucose decreases even with the once daily injection of insulin glargine. Nocturnal hypoglycaemia can be prevented with bedtime snacks. If a patient wishes to leave out bedtime snacks, this has to be tested individually.

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Comparison Between Different Regimens of Basal Insulin Supplementation in the Prevention of Nocturnal Hypoglycemia in Intensive Treatment of T1 DM

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To compare different options of replacement of basal insulin in T1 DM, 29 T1 DM patients on intensive treatment were randomized to receive either: i) four daily NPH administrations (at each meal, in combination with insulin lispro, and at bed-time, $N=10$); ii) glargine once daily at dinner-time ($N=10$), and iii) continuous subcutaneous insulin infusion (CSII, $N=9$) for four weeks. All groups received lispro insulin at meal-time. During the last week of treatment, the patients were monitored overnight on two occasions, after either the usual dose of basal insulin, or, 2-4 days apart, after a 15% increase in the dose of night-time basal insulin aiming at normal fasting plasma glucose (PG). On the first occasion, with NPH, plasma insulin peaked at 02:30 h to 192 ± 13 pmol/l and then decreased to 64 ± 4 pmol/l at 08:00 h. PG initially decreased to a nadir of 6.1 ± 0.6 mmol/l at 03:30 h, and then increased to 8.2 ± 0.7 mmol/l at 08:00 h. Three patients had asymptomatic hypoglycemia (PG < 4.0 mmol/l) between 02:00 and 04:30 h. After the usual dinner-time glargine dose and usual rate of CSII, overnight plasma insulin and PG remained nearly flat, no patient had hypoglycemia, and at 08:00 h PG was 7.3 ± 0.4 and 7.2 ± 0.2 mmol/l, respectively ($p < 0.05$ vs NPH). On the second occasion, after the 15% increase in NPH dose, PG decreased to a nadir of 4.6 ± 0.7 mmol/l between 04:00 and 05:00 h, and was 6.9 ± 0.9 mmol/l by 08:00 h. In 7 patients glucose had to be infused from 02:00 to 07:30 h to prevent PG < 2.8 mmol/l. With the 15% increase in the dose of glargine and rate of CSII, PG decreased between 24:00-08:00 h from 7.1 ± 0.4 to 5.8 ± 0.2 mmol/l and from 6.2 ± 0.2 to 5.6 ± 0.2 mmol/l, respectively ($p=NS$). No patient had hypoglycemia. Intra-patient coefficient of variation (CV) of plasma insulin was lower in both study occasions with glargine ($12 \pm 3\%$ and $10 \pm 2\%$) and CSII ($8 \pm 2\%$ and $10 \pm 2\%$) vs NPH ($32 \pm 2\%$ and $27 \pm 4\%$) ($p < 0.05$), as it was the CV of PG (glargine $7 \pm 1\%$ and $10 \pm 1\%$, CSII $7 \pm 1\%$ and $10 \pm 1\%$, NPH $15 \pm 4\%$ and $23 \pm 2\%$) ($p < 0.05$, glargine and CSII vs NPH). It is concluded that s.c. NPH insulin is a suboptimal approach as compared to glargine insulin and CSII to near-normalize PG in the post-absorptive state because of the high risk for nocturnal hypoglycemia. S.c. glargine insulin and CSII appear similarly suitable approaches to intensify blood glucose control in the post-absorptive state of T1 DM.

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KINETICS AND METABOLITE PROFILE OF INSULIN GLARGINE (LANTUS®)

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Background and Aims: Insulin glargine, a once-daily depot insulin, provides a basal insulin supply for 24 hours following subcutaneous injection. Hexamer complexes at the injection site gradually dissolve, the soluble monomer is absorbed. Kinetics and metabolism were studied in mouse, rat, rabbit, dog and human. **Materials and Methods:** Animals were treated by intravenous and subcutaneous injections. Insulin glargine and active metabolites were measured by RIA using a specific human insulin antibody with 56% cross-reactivity for insulin glargine (M0) and 47-55% for its active metabolites (M1, M2). In some studies, free insulin glargine was measured after removing endogenous antibodies by precipitation. Insulin antibodies were determined by a validated tracer-binding test. **Results:** After i.v. injection, insulin glargine disappeared rapidly from the circulation similar to regular human insulin. After s.c. injection, ¹²⁵I-labelled insulin glargine showed a significantly delayed elimination, up to 24 hours, depending on species. The time course was closely correlated with the pharmacodynamics of blood glucose lowering activity. In repeated dose studies in dogs, there was no accumulation of insulin glargine, and no antibody formation. *In vitro* and *in vivo* metabolism studies indicated that degradation proceeds via C-terminal cleavage of the [B31, B32] arginines followed by cleavage of the [B29-Lys, B30-Thr] bond. Degradation products detected in the various studies were [A21-Gly, B31-Arg]-insulin, [A21-Gly]-insulin (M1) and [A21-Gly, B30-(desThr)]-insulin (M2). Parent compound and metabolites M1, M2 are biologically active. Plasma of rats and dogs contained more degradation products than human plasma. Active immunisation studies in animals showed lower or similar immunogenic potential than human, porcine and bovine insulin. **Conclusion:** Insulin glargine has a slower absorption and prolonged duration of action than conventional insulin depot preparations. Metabolites are closely related to human insulin.

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Extremely Prolonged Action of the Dicarboxylic Acid Acylated Insulin Analog O346 after Intravenous Injection in Dogs

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Background and Aims: Development of soluble human insulin analogues with a very prolonged and reproducible action is crucial for the provision of stable basal insulin and normalized glucose homeostasis in diabetes management. In the present study we examined metabolic effects and plasma and interstitial fluid (ISF) kinetics of fatty acid acylated insulin, Lys^{B29}(N^{epsilon}-omega-carboxy-nonadecanoyl)-desB30 human insulin (O346) an analog of the B29 lysine acylated family with very high albumin binding affinity (36×10^5 M⁻¹; 37°C).

Materials and Methods: Euglycaemic clamps with somatostatin (0.8 mg/min per kg) and basal replacement insulin (0.2 mU/min per kg) infusion were carried out in 13 male mongrel dogs under inhalant anaesthesia. 3-3H-D-glucose was infused to determine glucose turnover. Saline control ($n=3$), or intravenous injection of O346 (15U/kg, $n=8$) experiments were performed for a period of 8h. Plasma and ISF insulin concentration data was fit to a 2 compartment (plasma and ISF) model.

Results: After intravenous injection a $t_{1/2}$ for the clearance of O346 from plasma of 420 min and a $t_{1/2}$ for the appearance of O346 in ISF of 150 min was measured. Glucose disposal was well correlated with the ISF O346 concentration ($r=0.87$, $p < 0.001$) as previously reported for native insulin as well as analogs with affinity for albumin less than O346 (e.g., NN304). Glucose disposal with O346 was increased 4-fold over basal at $t=480$ min compared to pre-injection ($t=0$ min) or saline control ($t=480$ min). O346 Plasma elimination and transendothelial transport was 0.2% and 3.5% of regular insulin 2% and 50% of NN304. Combination of *in vivo* results and compartmental modelling suggest that O346 has about 20% of the potency of regular insulin in dogs. Maximal stimulation of glucose disposal was estimated to occur 10h after intravenous injection of the analog.

Conclusions: This study demonstrates that the dicarboxylic acid acylated insulin analog O346, with very high binding affinity to albumin, is active in dogs. The compound acts very slowly, but its effect on glucose disposal rate can be maintained for at least 8 hours. O346 kinetics suggests that the plasma compartment acts as a storage compartment for O346 from which the analog is slowly released to insulin sensitive tissues. The reason for the lower potency of the analog remains to be elucidated.

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Short-Acting Insulin Analogues

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A RANDOMIZED TRIAL OF INSULIN ASPART WITH INTENSIFIED BASAL NPH INSULIN SUPPLEMENTATION IN TYPE 1 DIABETES
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Background and Aims: Insulin aspart (aspart), a rapid-acting insulin analogue, has been shown to result in improved postprandial glycaemic control. We tested the hypothesis that aspart combined with intensified basal NPH insulin supplementation results in improved overall glycaemic control, compared to human insulin and standard NPH insulin. **Materials and Methods:** People with type 1 diabetes were randomized to 15 months treatment with aspart (80% of previous human insulin dose) as mealtime insulin with additional NPH doses if meals were >5 hours apart and a 25% increase in bedtime NPH (n=187), or human insulin with once or twice daily NPH insulin (n=181). Efficacy and safety were evaluated at 3 months (primary evaluation period) and 15 months. **Results:** An algorithm-driven reduction in mealtime insulin dose by 13% and an increase in basal insulin dose by 35% resulted in a non-significantly (p=0.22 and p=0.13) lower HbA_{1c} in the aspart group (-0.09%, 95%CI -0.23% - +0.05%, and -0.14%, 95%CI -0.32% - +0.04%, at 3 and 15 months). Postprandial glucose values at 3 months were lower in the aspart group than in the human insulin group: post-breakfast 8.27 ± 0.32 vs 9.31 ± 0.32 mmol/l (\pm SEM) (p=0.0137), post-lunch 7.4 ± 0.25 vs 8.37 ± 0.25 (p=0.0037), post-dinner 8.22 ± 0.28 vs 8.87 ± 0.28 (p=0.081). The differences at 15 months were similar. No significant differences were found in hypoglycaemia or adverse event rate. There was no difference in treatment satisfaction (WHO Diabetes Treatment Satisfaction Questionnaire) and health related quality of life (Diabetes Health Profile) at three months, but at 15 months treatment satisfaction was higher in the aspart group: difference 1.57 points (95%CI 0.49-2.64, p=0.004). **Conclusions:** Improved postprandial glycaemic control and treatment satisfaction with insulin aspart were confirmed. Intensified basal insulin supplementation resulted in a similar HbA_{1c} decrement as previously found with aspart and NPH insulin once or twice daily.

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The Effect of Insulin Aspart on Postprandial Metabolism and Metabolic Outcome in Type 2 Diabetes.

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Background and Aims: Type 2 diabetes is associated with a wide variety of metabolic and haemostatic abnormalities including dyslipidaemia, raised albumin excretion rate (AER), excessive fibrinogen and low fibrinolytic activity with high plasminogen activator inhibitor 1 (PAI-1) levels. Elevated markers of endothelial dysfunction such as E-selectin and high levels of homocysteine are also thought to contribute to the adverse risk of arterial disease in Type 2 diabetes. We aimed to examine whether the restoration of a more physiological insulin profile, using rapid-acting insulin aspart to reduce postprandial hyperglycaemia, would have any effect on the metabolic and haemostatic abnormalities in Type 2 diabetic patients.

Materials and Methods: Insulin-treated Type 2 diabetic patients (n=21), mean age 66 ± 1 yr, BMI 30 ± 0.5 kg/m², duration of diabetes 11 ± 1 yr, were recruited into a single centre, randomized, crossover, double-blind study. After a 4-week run-in period patients were randomized to unmodified human insulin or insulin aspart before main meals, both with basal NPH, for 6-week study periods. Treatment targets were 4.0-6.0 mmol/l preprandially and 5.0-7.5 mmol/l postprandially in the absence of hypoglycaemia. At the end of each 6-week treatment period metabolic control was assessed and a test meal study performed. Fasting levels of metabolic parameters were measured and AER was estimated using the results of three timed overnight urine collections. Patients were crossed over to the alternative pre-meal insulin for a further 6 weeks.

Results: There was no difference in HbA_{1c} (mean \pm SE: 7.04 ± 0.13 vs 7.15 ± 0.11 %, p=0.060) or fructosamine (362 ± 9 vs 371 ± 13 μ mol/l, p=0.312) with insulin aspart compared to unmodified human insulin. Postprandial blood glucose at 90 min was lower with insulin aspart than with unmodified human insulin (8.4 ± 0.5 vs 9.2 ± 0.6 mmol/l, p=0.046). No significant differences occurred in fasting levels of total cholesterol, HDL-cholesterol, triglyceride, apolipoprotein A1, apolipoprotein B, fibrinogen, PAI-1, E-selectin, or homocysteine between the two groups. AER was only significantly reduced (median(range): $4.7(2-2473)$ vs $8.0(2-3298)$ μ g/min, p=0.039) in patients with improved postprandial control on insulin aspart (n=15).

Conclusions: Insulin aspart use thus resulted in improved postprandial glycaemic control when compared to human insulin in Type 2 diabetic patients. This was associated with reduced AER, but was without changes in other markers of vascular risk.

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INSULIN ASPART SAFE FOR LONG-TERM TREATMENT

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Background and Aims: Previous attempts to improve glycaemic control with intensive therapy of type 1 diabetes have been associated with a three-fold increase in severe hypoglycaemia. Intensive therapy with the rapid acting insulin analogue, insulin aspart, has been shown to be associated with a small reduction in HbA_{1c} over 6 months compared with human soluble insulin without any adverse impact on major hypoglycaemia. We hereby report on the efficacy and safety of insulin aspart in a three-year study. **Materials and Methods:** The study was a multi-centre, open-labelled, parallel-group, 2½ year extension study in adult patients with type 1 diabetes. In the initial six-month trial patients were randomised 2:1 to insulin aspart or unmodified human insulin before meals, with NPH-insulin as basal insulin. 753 subjects continued their allocated treatment in the extension trial. Efficacy and safety were evaluated for 2½ years of exposure. The main outcomes measured were hypoglycaemia, classified as major (requiring third party intervention) or minor, adverse events, and blood glucose control as assessed by HbA_{1c}. The relative risk of major hypoglycaemic episodes was estimated from a comparison of the frequencies of episodes with insulin aspart and human insulin in a longitudinal generalised linear Poisson regression model. HbA_{1c} was compared using a repeated measures ANOVA model. **Results:** Mean HbA_{1c}, adjusted for baseline, country and total daily dose, was 0.17 absolute percentage points lower with insulin aspart than with human insulin (95% CI -0.32 to -0.02, P=0.028). The risk of experiencing a major hypoglycaemic episode with insulin aspart was the same as with human insulin: The relative risk was 1.00 (95% CI: 0.72-1.39, NS). The relative risk of experiencing a minor hypoglycaemic episode was reported to be 1.24 with insulin aspart: (95% CI: 1.09-1.39, P = 0.02). Only 1% of the subjects in each treatment group withdrew due to adverse events; relation to trial drug was unlikely in all cases. Overall, more patients on human insulin than on insulin aspart withdrew from the study: 59 (32% of subjects) vs. 96 (17% of subjects). **Conclusions:** Insulin aspart was proven safe for long-term use. HbA_{1c} was maintained with insulin aspart without any adverse impact on the rate of major hypoglycaemia.

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INSULIN ASPART EFFICACY AND SAFETY COMPARED TO HUMAN SOLUBLE INSULIN AND HUMAN PREMIX INSULIN (30/70) IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Aims: The aim of the present study was, in an open-labelled, randomised, 3-arm parallel-group design including a 1-3 weeks titration period and a 3 months maintenance period to compare in patients with OHA or premix insulin treated type 2 diabetes the efficacy and safety of (1) preprandial insulin aspart (IAsp) (with or without bedtime NPH insulin), (2) preprandial human soluble insulin (HI) (with or without bedtime NPH insulin), (3) with human premix insulin (MIX) (once or twice daily).

Patients and Methods: 231 (130 M/ 101 F) type 2 patients from 30 centres with a mean age of 62.2 (SD 8.8) years, Body Mass Index (BMI) 29.3 (SD 3.6) kg/m², HbA_{1c} 7.81 (SD 1.12) %, diabetes duration 10.2 (SD 7.3) years were randomised to either IAsp (n=75), HI (n=80) or MIX (n=76), 204 patients completed the trial according to the protocol. HbA_{1c}, 7-point blood glucose (BG) profiles, fasting blood glucose, insulin dosage, hypoglycaemic episodes, adverse events and standard safety parameters were measured. The primary efficacy endpoint was the change of HbA_{1c} from baseline to the last visit. Analysis for equivalence was performed by T-tests with alpha = 0.83% for each of 3 subtests.

Results: Equivalence with regard to HbA_{1c} between the 3 therapies could not be demonstrated. HbA_{1c} decreased by 0.91% (SEM 0.12) in the IAsp group, by 0.73% (SEM 0.10) in the HI group and by 0.65% (SEM 0.13) in the MIX group. Postprandial BG levels decreased most pronounced in the IAsp group: 8 to more than 30 mg/dl when compared to the HI group, and 20 to more than 30 mg/dl when compared to the MIX group. The mean preprandial insulin doses per injection were similar in the IAsp (10-13 units) and the HI group (10-14.5 units). The hypoglycaemia ratio/month exposed was 0.56 in the HI group, 0.40 in the IAsp group and 0.19 in the MIX group.

Conclusion: Equivalence could not be shown between the 3 therapies. However, in this study population IAsp lead to better glycaemic control in terms of HbA_{1c} and postprandial BG levels when compared to HI and MIX. The treatment with IAsp revealed to be very safe and well tolerated.

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Insulin aspart reduces the frequency of nocturnal hypoglycaemia in patients with Type 1 diabetes

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Background and Aims: Trials with rapid-acting insulin analogues in basal bolus regimens have generally produced modest reductions in the rate of hypoglycaemic events and have been criticised for not being blinded. Previous non-blinded studies with insulin aspart have indicated a reduction in the rate of night time hypoglycaemic events. This double-blind, randomised, crossover trial compared the rapid-acting analogue insulin aspart (IAsp) and soluble human insulin (HI) with respect to the rate of hypoglycaemic events in an intensive basal-bolus setting.

Materials and Methods: A total of 155 patients with Type 1 diabetes were symmetrically randomised to two 16-week treatment periods on either IAsp or HI, both injected 0-5 minutes before meals. The treatment period was preceded by a 6 week run-in period. During the treatment periods, NPH insulin was given as basal insulin once or twice daily as needed, and insulin dosages were adjusted regularly using predefined algorithms to maintain tight glycaemic control. Treatment periods were separated by a 4 week wash-out period.

Results: The rate of major (requiring third-party intervention) nocturnal hypoglycaemic events (2400-0600h) was substantially reduced with IAsp treatment compared with HI treatment (0.017 v 0.056 events/month, $p < 0.005$). Total major hypoglycaemic event rates tended to be lower with IAsp (0.071 v 0.093 events/month, NS). Furthermore, there were fewer minor hypoglycaemic events with IAsp treatment (2.98 v 3.19, $p < 0.05$). No differences were found in HbA1c following the two treatments.

Conclusions: IAsp compared with HI showed a marked 72% reduction in the relative risk of experiencing a major nocturnal hypoglycaemic event and also a significant reduction in the rate of minor hypoglycaemic episodes. This reduction was achieved with maintained glycaemic control. Thus these data demonstrate a clinically significant advantage of using insulin aspart in basal-bolus treated patients with Type 1 diabetes.

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EQUIVALENT GLYCEMIC CONTROL WITH PRE-MEAL OR POSTMEAL HUMALOG® MIX25™ IN ELDERLY PATIENTS WITH TYPE 2 DIABETES.

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Background and Aims: Postmeal insulin injections could be a useful option for elderly patients who forget a pre-meal injection or whose eating habits are unpredictable. We compared the effects on glycaemic control of pre-meal injections of Humalog Mix25 (Mix25; 25% insulin lispro, 75% NPL), known as Humalog Mix75/25™ in the US, to those of postmeal injections of Mix25 in elderly patients with type 2 diabetes and inadequate control with sulfonylurea (HbA1c >1.2-fold of upper limit of the normal range).

Materials and Methods: This open-label, 16-week, parallel group study randomized patients (60 to 80 years old) into one of two groups: Mix25 injected either immediately before (n=37) or within 15 minutes after (n=35) the start of the breakfast and evening meals. Mean HbA1c, blood glucose (BG), and body weight in the two groups were not significantly different at baseline.

Results: Endpoint HbA1c was similar in the pre-meal and postmeal groups (8.54±0.22% [mean±SEM] vs. 8.74±0.26%, $P=0.557$). At endpoint, the daily BG profile showed these mean BG values (±SEM) (mM) for pre-meal and post-meal injection, respectively: fasting, 8.27±0.34 and 8.07±0.31, $P=0.673$; 2 hr after breakfast, 9.32±0.44 and 10.13±0.60, $P=0.280$; pre-dinner, 9.72±0.52 and 9.81±0.40, $P=0.897$; 2 hr after evening meal, 9.79±0.42 and 10.45±0.47, $P=0.386$; overall daily BG, 9.27±0.42 and 9.61±0.39, $P=0.555$. Mean insulin doses in the pre-meal and postmeal groups were 0.42±0.03 U/kg and 0.51±0.03 U/kg, respectively ($P=0.033$). The endpoint hypoglycaemia rate (symptoms or BG<3mmol/L) was low (0.56±0.42 episodes/30 days vs. 0.06±0.04 episodes/30 days, $P=0.236$). The groups did not differ significantly in body weight change from baseline to endpoint ($P=0.727$).

Conclusions: We conclude that in an elderly population, the injection of Mix25 either pre-meal or postmeal resulted in comparable overall glycaemic control (HbA1c) with a low frequency of hypoglycaemia. The postmeal injection of Mix25 is a safe therapeutic option that may be particularly useful for elderly patients with type 2 diabetes.

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Impact of conversion to insulin lispro therapy in obese patients with diabetes mellitus type 2 - an observational trial

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Background and Aims: A couple of new treatment opportunities for the treatment of type 2 diabetes have been developed during the recent years. This uncontrolled prospective observational study was performed to gain experience regarding the efficacy and safety of prandial insulin lispro treatment in obese patients with type 2 diabetes mellitus under routine conditions.

Materials and Methods: 4931 patients treated by 1087 physicians were included (2433 male, 2498 female; mean age 59.6±10.8 years (mean±SD); mean duration of diabetes 8.8±5.7 years; mean body mass index 31.2±4.6 kg/m²). Patients were observed for a mean duration of 50.1±30.2 days.

Results: Self measured blood glucose values improved significantly at all time points during the day ($p < 0.001$) with a mean reduction of highest blood glucose values from 16.2 ±3.8 to 11.1±2.3 mmol/l ($p < 0.001$). Mean HbA1c levels decreased from 9.2±1.5 before starting insulin lispro to 7.9±1.2 % at the end of the observational period ($p < 0.001$). During the 4 weeks before starting insulin lispro, hypoglycaemic episodes had been reported in 132 patients (mean incidence: 2.1 episodes per patient); during the first 4 weeks after start of insulin lispro in 66 patients (mean incidence: 1.5 episodes per patient) ($p < 0.001$). Mean body weight declined from 89.4±14.9 kg before conversion to insulin lispro to 88.2±14.7 kg at the end of the observational period ($p < 0.001$).

Conclusions: Switching to prandial insulin lispro therapy in obese patients with diabetes mellitus type 2 improves metabolic control without increasing body weight or hypoglycaemic events. The results of this observational trial, performed under routine conditions, confirm the beneficial effects of insulin lispro in type 2 diabetes observed in previous well controlled clinical studies.

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BIPHASIC INSULIN ASPART AND BIPHASIC HUMAN INSULIN COMPARED IN TYPE 2 DIABETIC SUBJECTS.

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Background and Aims: The long-term safety of biphasic insulin aspart 30 (BIAsp 30) was compared with a similar biphasic formulation of human insulin (BHI 30). The mixtures consisted of 30% soluble and 70% protamine-bound insulin. Rates of major hypoglycaemia and adverse events, antibody formation and clinical laboratory parameters (CLP) were addressed.

Materials and Methods: This was part of a 24-month randomised, open-label parallel group study comparing BIAsp 30 and BHI 30 taken twice daily before meals. 125 subjects with Type 2 diabetes (58 in the BIAsp 30 and 67 in BHI 30 group) entered the trial. Major (requiring third party help) and minor (self treated) hypoglycaemic episodes were recorded from subject diaries. Antibody formation and CLPs were followed during treatment.

Results: Ninety-five patients (76%) completed the 2-year treatment period. The frequency of major hypoglycaemic episodes was low, and decreased during the trial. First treatment year: Four episodes in the BIAsp 30 and 11 in the BHI 30 group. Second year: Zero and 8 episodes, respectively in the two groups ($p = 0.02$). Minor episodes were also fewer in the BIAsp 30 group, especially in the second treatment year ($p = 0.05$). The number of adverse events (AEs) was similar in the two groups. Drug related AEs, Serious AEs, and diabetes related AEs were few and similar in both groups. Cross-reacting insulin antibodies increased initially, decreased later and were not statistically different in the treatment groups at end of trial. Mean values and standard error of means (SEM) were: 14.8 (3.1) in the BIAsp 30 group and 11.6 (2.5) in the BHI 30 group at end of trial. CLPs remained normal and stable in both treatment groups throughout the trial. Glycaemic control as assessed by HbA1c did not differ between groups: mean and (SEM) were 8.25 (0.14) in the BIAsp 30 group and 8.21 (0.13) in the BHI 30 group at 24 months.

Conclusions: These results confirm the long-term safety and efficacy with BIAsp 30 treatment in Type 2 diabetes. Over a 2-year period, the frequency of major episodes was lower than after treatment with BHI 30.

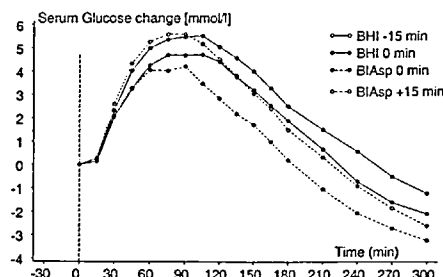
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POSTPRANDIAL DOSING OF THE "LOWMIX" BIPHASIC INSULIN ASPART

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Background and Aims: We compared the effect of postprandial dosing of biphasic insulin aspart 30 (BIAsp) with preprandial or prandial dosing of BIAsp or Biphasic Human Insulin 70/30 (BHI) on glucose excursions. **Materials and Methods:** Thirtyone type 2 diabetic patients (57±6 years; 21 men; BMI 29±4 kg/m²; HbA_{1c} 8.7±1.3% (mean±SD)) received a standard breakfast in an open-label randomized four-period-crossover study. Postprandial BIAsp_{+15min} was compared with prandial BIAsp_{0min}, BHI_{0min} and preprandial BHI_{15min}. Insulin doses were chosen individually but were kept constant in each patient. **Results:** Serum glucose AUC corrected for baseline over 5 h showed significantly lower values with BIAsp_{0min} compared to BIAsp_{+15min} (ratio 1.11, 95%CI: 1.04-1.18; p<0.01 (ANOVA)). Postprandial BIAsp_{+15min} was comparable to prandial BHI_{0min} (ratio 0.95, 95%CI: 0.89-1.02; NS) and preprandial BHI_{15min} (ratio 0.99, 95%CI: 0.93-1.05; NS). **Conclusions:** Postprandial injection of biphasic Insulin Aspart as a new treatment option offers more flexibility to type 2 diabetic patients and does not impair postprandial blood glucose concentrations compared to human insulin.



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Oral and Pulmonary Insulin Delivery

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INHALED INSULIN WITH AN IMPROVED SPIROS® DRY POWDER INHALER: DOSE-RESPONSE AND TIME-ACTION PROFILES

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Background and Aims: This euglycaemic glucose-clamp study investigated the pharmacokinetics (PK), pharmacodynamics (PD), safety and tolerability of human insulin inhalation powder (HIIP) delivered by an Spiros® dry powder inhaler system in healthy subjects. **Materials and Methods:** Twelve healthy, non-smoking volunteers (age 30±7 y; BMI 23.5±2.7 kg/m²; mean±SD) with normal pulmonary function (FEV₁ and FVC) participated in an open-label, randomised, 6-period crossover trial. Each subject inhaled (INH) 4 different doses of HIIP (2, 3, 4, and 5 blisterwells (B)) on separate occasions. For comparison, 2 doses of subcutaneous (SC) regular human insulin were injected (8, 14, or 20 U). **Results:** On average, serum insulin with inhaled insulin peaked 60 min earlier (insulin T_{max}; median (range)) than with SC injected insulin. Following inhalation GIR_{max} peaked 70 min earlier than with SC insulin (p<0.04). The dose response relationships of PK and PD were linear and were similar between inhaled and SC insulin. Dosing was well tolerated in all subjects.

	insulin C _{max} (μU/ml)	insulin T _{max} (min)	GIR _{max} (mg/kg/min)	GIR T _{max} (min)
8 U SC	31±7	120 (90–150)	4.1±1.3	227±153
14 U SC	34±11	105 (30–300)	5.1±2.7	241±73
20 U SC	59±22	120 (20–150)	6.1±2.5	241±29
2 B INH	24±11	30 (10–90)	2.0±0.8	187±136
3 B INH	31±14	20 (10–150)	3.3±1.8	129±86
4 B INH	38±17	45 (10–120)	3.4±1.7	161±129
5 B INH	46±22	20 (10–240)	4.2±1.9	162±118

Conclusions: This study demonstrated that oral inhalation of human insulin via the Spiros® DPI provides a promising and safe alternative method for administration of human insulin.

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TIME ACTION PROFILE OF INHALED INSULIN VIA SPIROS DRY POWDER INHALER IS CONSISTENT AMONG USER INHALATION TECHNIQUES

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Background and Aims: This study investigated the impact of breath-hold (BH) and inhalation duration (I) on the effect of insulin delivered via a novel breath-actuated, palm-sized, aerosol system that administers insulin upon inspiration, independent of the inspiratory flow rate. The Spiros DPI system is designed to deliver consistent insulin dosing independent of user inhalation technique.

Materials and Methods: The euglycaemic clamp technique was used to define the time-action profile following inhaled human insulin in a group of 6 healthy nondiabetic subjects aged 24±3 yrs with a BMI of 23±1.7 kg/m². The study used a randomized crossover design where each subject received a single dose of inhaled insulin (2.31 mg emitted) on 4 occasions. Standard dosing technique, I for 6 secs and BH for 10 secs (I6BH10); alternative conditions tested were I6BH0, I6BH20, and I3BH10. Doses were given after a 10-hour fast, followed by a 10-hour glucose clamp. Samples were collected for insulin and C-peptide. Pharmacokinetic (PK) parameters were derived from serum insulin concentration vs time profiles. Pharmacodynamic (PD) parameters were derived from glucose infusion rate vs time profiles.

Results: There were no statistically significant differences in PK or PD parameters between the various inhalation techniques. Standard dosing technique showed a profile of early action reflected in both PK and PD parameters. PK: mean peak insulin concentration (C_{max}) 1.13±0.59 ng/mL; mean time of peak (T_{max}) 30 (15–90) min. PD: time of onset of insulin action (tonset) 16 (10–53) min; peak glucose infusion rate (R_{max}) 235±92 mg/min; time to peak (TR_{max}) 112 (42–240) min; time to 50% peak (TR_{max/2}) 35 (18–51). Dosing was well tolerated in all subjects.

Conclusion: Inhalation of human insulin via the Spiros DPI produced a favorable time-action profile. Wide variations of inhalation duration and breath-hold parameters did not impact this profile, demonstrating consistent performance of the inhaler system and supporting further development of this promising drug delivery system.

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Influence of small dose i.v., s.c. and pulmonary insulin treatment on prandial glucose control in patients with Type 2 diabetes

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Background and Aims: The lack of the first phase insulin release is one of the first deteriorations in Type 2 diabetes. The goal of this study was to explore the effect of a restoration of this first phase release with small doses of preprandial insulin on postprandial glucose control using i.v. insulin treatment as comparator.

Materials and Methods: This open randomized four-way crossover study was conducted with 12 patients (2 female, 10 male, mean(±SD): age: 62(6) yrs, duration of diabetes 10.5(8.3) yrs (range 2 - 21 yrs)). Before the uptake of a standardized meal, the patients received small doses of insulin formulations with a known fast onset of action: 3 IU of i.v. regular insulin (Tmax: ~10 min), 6 IU of s.c. insulin lispro (Tmax: ~45 min), and 12 IU, or 24 IU of pulmonary Technosphere/Insulin (Tmax: ~15 min). Observation parameters for the following six hours after the meal were: blood glucose, insulin, C-peptide, proinsulin, glucagon and free fatty acids.

Results: The patients reacted differently to the intravenous therapy. While postprandial glucose values stayed below 180 mg/dl in five patients (mean maximal value: 155.6 (30.2) mg/dl, responders, R), they exceeded this arbitrary limit in the other seven patients (210.1 (49.3) mg/dl, p<0.01, Non-responders, NR). A tendency for higher pancreatic secretion as indicated by higher second phase insulin, proinsulin and C-peptide concentrations was observed after about 3 h in the NR group (4h values, insulin NR: 25.6 (8.6) µU/ml, R: 16.5 (14.1) µU/ml (n.s.); proinsulin NR: 63.9 (24.1) pMol, R: 42.5 (16.0) pMol (p=0.116; p<0.05 at 6h), C-peptide NR: 4.8 (1.6) ng/ml, R: 3.7 (2.3) ng/ml, n.s.). The free fatty acid levels as well as the glucagon concentrations tended to be more elevated in the NR group during the entire experiment. No significant differences were seen in any of the observation parameters when the i.v. treatment was compared to any of the other three treatment arms with fast acting s.c. or pulmonary insulin formulations.

Conclusions: In this exploratory pilot study it could be shown that preprandial insulin treatment with small doses of fast acting insulin formulations may be a suitable treatment for at least a subgroup of patients with Type 2 diabetes, most likely those in an earlier stage of the disease. Further research work is required to investigate the clinical specifications of the patients and the optimal therapeutic approach. Given the pharmacokinetic profile and the convenience of application, pulmonary Technosphere/Insulin may be an attractive candidate for this type of treatment.

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INTRA-SUBJECT VARIABILITY OF PULMONARY INSULIN VIA THE AERx[®] INSULIN DIABETES MANAGEMENT SYSTEM VERSUS SUBCUTANEOUS INSULIN

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Background and Aims: This trial was aimed to compare the intra-subject variability in postprandial serum insulin (INS) and serum glucose (GLU) concentrations after pulmonary administration of an aerosol of liquid human insulin via the AERx[®] iDMS (insulin Diabetes Management System) and after regular s.c. insulin. **Materials and Methods:** In this parallel, randomised, open-labelled trial 17 non-smoking male Type 1 diabetics (mean age 28 years [range 21 - 38], HbA_{1c} 7.7% ± 0.8% [mean ± SD]) received a standard breakfast on four identical treatment days. The subjects received their usual insulin dose either as pulmonary insulin via a handheld AERx[®] iDMS (immediately before the meal, 9 subjects) or as s.c. insulin injection (30 minutes before the meal, 8 subjects). INS and GLU were measured predose and regularly during six hours postdose. **Results:** There was no statistically significant difference in the intra-subject variability for any of the INS and GLU variables investigated (see table). AUC_{0-360, INS} for pulmonary insulin was 135 ± 46 mU/L * hour vs. 143 ± 39 mU/L * hour for s.c. insulin. Adverse events were few and mild, and no clinically relevant deterioration in pulmonary function was detected. **Conclusion:** The intra-subject variability in serum insulin and serum glucose concentrations for pulmonary insulin was comparable to s.c. insulin. Pulmonary insulin delivery was safe and well tolerated in Type 1 diabetic subjects.

End point	Intra-subject Coefficient of Variation (CV) (%)				
	Pulm. INS	S.c. INS	Ratio (P/S)	95% C.I.	P-value
AUC _{INS}	24%	20%	1.20	0.66; 2.06	0.54
C _{max, INS}	23%	22%	1.03	0.64; 1.62	0.90
AUC _{GLU}	30%	23%	1.30	0.84; 1.98	0.23

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DOSE-RESPONSE OF PULMONARY INSULIN WITH THE AERx[®] INSULIN DIABETES MANAGEMENT SYSTEM IN HEALTHY SUBJECTS

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Background and Aims: This study was aimed to investigate the pharmacokinetic (PK) and pharmacodynamic (PD) dose response relationship of a liquid pulmonary insulin aerosol applied by means of the AERx[®] iDMS (Insulin Diabetes Management System). **Materials and Methods:** Seventeen non-smoking, healthy male subjects (mean age 29.9 years [range 23 to 37]; BMI median 25.0 [range 18.0-29.9]) received 2, 4, 6 and 8 AERx[®] units (one AERx[®] unit being approximately equivalent to one s.c. unit) and 6 IU of s.c. soluble insulin on 5 study days in this euglycaemic glucose clamp study (clamp level 5 mmol/L, clamp duration 10 hours after drug administration). **Results:** Insulin endpoints were derived from measured insulin concentrations. GIR endpoints were derived from total GIR profiles. A clear dose response relationship was demonstrated for both PK and PD characteristics across the four different pulmonary insulin doses; the time to maximal action was not affected by the dose. No drug-related adverse events were observed. **Conclusions:** Pulmonary insulin delivery with the AERx[®] iDMS showed a clear dose-response relationship. Insulin administration was convenient, safe and well tolerated in healthy subjects.

Insulin dose	AUC _{0-10h} (mU/L*h)	C _{max,INS} (mU/L)	AUC _{GIR} (0-10h) (g/kg)	GIR max (mg/kg*min)
AERx U 2	168 [156;235]	29.6 [23.5;32.1]	2.17 [1.40;2.91]	6.35 [4.10;8.54]
AERx U 4	162 [139;237]	33.1 [23.7;41.2]	2.31 [1.61;2.61]	6.90 [4.20;8.83]
AERx U 6	210 [163;253]	40.3 [31.3;46.8]	2.60 [2.23;3.21]	8.12 [6.50;9.92]
AERx U 8	236 [200;303]	47.1 [37.3;59.8]	3.13 [2.12;3.48]	8.70 [6.68;11.1]
s.c. 6 IU	204 [173;279]	33.3 [28.5;42.7]	2.59 [2.24;2.69]	7.75 [6.67;9.46]

Numbers are median [1st - 3rd quartile]

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REPRODUCIBILITY OF INHALED AND SUBCUTANEOUS INSULIN IN TYPE 2 DIABETIC PATIENTS

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Background and Aims: Dose-to-dose reproducibility is a crucial component of insulin therapy. We compared the reproducibility of the pharmacokinetic (PK) and pharmacodynamic (PD) responses to inhaled insulin (INH) delivered via the Aerodose[™] Inhaler to that of subcutaneous regular insulin (SC) in Type 2 diabetic patients under glucose clamp conditions. Such a comparison of INH and SC has not been previously reported. **Materials and Methods:** Fifteen patients (non-smokers, 10 male, 5 female, age 60±9 years, [mean±SD], BMI 30±3 kg/m², diabetes duration 9±4 years) each received two doses of 240 U INH and two doses of 24 U SC on 4 separate study days under euglycaemic glucose clamp conditions (180 min baseline, clamp level 6.1 mmol/L, continuous i.v. insulin infusion at 0.3 mU/kg/min). Glucose infusion rates (GIR) and serum insulin concentrations were monitored over the following 8 h. Comparisons of intra-patient coefficients of variation (CV) were used to assess the reproducibility of INH vs SC at 0-3 h (the anticipated time of meal coverage) and 0-8 h. **Results:** INH showed a relative bioavailability (0-8h) of 16% and relative biopotency of 13%. There were no significant differences between INH and SC in the CVs for Insulin-AUC and GIR-AUC (p>0.2, table).

	Mean		Intra-patient CV, %	
	INH	SC	INH	SC
Insulin-AUC ₀₋₃ (mU·mL ⁻¹ ·min)	12.2±8.2	4.9±2.1	18.5	22.5
Insulin-AUC ₀₋₈ (mU·mL ⁻¹ ·min)	22.0±13.7	13.7±3.8	22.0	16.3
GIR-AUC ₀₋₃ (g/kg)	0.7±0.3	0.4±0.2	18.8	21.5
GIR-AUC ₀₋₈ (g/kg)	1.7±0.5	1.3±0.4	21.0	18.6

INH exhibited a significantly shorter time to peak insulin concentration (T_{max, INH}: 76±51 vs SC: 193±66 min, p<0.0001) and time to peak metabolic effect (T_{GIRmax}: 170±53 vs 244±75 min, p<0.0001) as compared to SC. No adverse events were observed during the study. **Conclusion:** The comparable dosing reproducibility vs SC and shorter time to peak action suggest that the Aerodose Inhaler may provide suitable pre-prandial dosing of insulin to Type 2 diabetic patients.

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PHARMACOKINETICS OF PULMONARY INSULIN IN HEALTHY SMOKERS AND NON-SMOKERS

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Background and Aims: The AERx[®] iDMS (insulin Diabetes Management System) is a potential diabetes treatment option for delivery of an aerosol of liquid human insulin to the deep lung for systemic absorption. The aim of this trial was to assess pharmacokinetics (PK) of pulmonary insulin in healthy smokers and non-smokers. **Materials and Methods:** On two consecutive days, 27 smokers and 16 non-smokers (18M/25F, mean age 26 y, BMI 23 kg/m²) received single doses of pulmonary insulin (33.75 U). Glucose infusion was given in case of hypoglycaemia. Results: PK results were derived from exogenous insulin profiles corrected for baseline C-peptide. Total insulin absorption ($AUC_{(0-6h)}$) was significantly greater in smokers, while peak concentration was higher and earlier in this group.

PK Variable	Non-smokers Mean (N=13)	Smokers Mean (N=23)	Mean Ratio	95% C.I.	p-value
$AUC_{(0-6h)}$ (mU/L·h)	40.0	63.2	1.58	1.20; 2.08	0.0017
C_{max} (mU/L)	13.9	42.0	3.02	1.94; 4.70	<0.0001
t_{max} (min)	53.9	31.5	-22.4	-34.2; -10.6	0.0003

Estimated means, ratios, confidence intervals (C.I.) and p-values based on ANOVA with log-transformed response, adjusted for sex and period; for t_{max} based on mean difference. Evaluable subjects: N=36.

No safety issues arose; adverse events were few and mild. Thirteen subjects received glucose infusion due to induced hypoglycaemia. Neither changes nor differences between groups were seen in lung function, vital signs or lab values. **Conclusions:** Absorption of pulmonary insulin was significantly greater in smokers, with a higher $AUC_{(0-6h)}$, a higher C_{max} and a shorter t_{max} . Dosing of pulmonary insulin with the AERx[®] iDMS was safe and well-tolerated.

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ORAL SPRAY INSULIN IN PATIENTS WITH TYPE 1 DIABETES: COMPARISON WITH REGULAR INSULIN

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BACKGROUND & AIMS: A new oral insulin spray formulation has been developed consisting of rapid insulin delivered by aerosol spray with a RapidMist device. Such a formulation allows insulin to enter the blood stream through buccal mucosa absorption. The aim of this study was to test the efficacy of oral spray insulin to control blood glucose levels in patients with Type 1 diabetes. **METHODS:** Patients with Type 1 diabetes (n=9) aged between 20-36 years (mean age 30 years) with a mean diabetes duration of 4.7 years and mean HbA1c of 6% were recruited in the study. This consisted of two days of follow-up comparing subcutaneous insulin treatment with oral spray insulin. On day 1 patients received their usual dose of subcutaneous regular insulin while on day 2 subcutaneous insulin was replaced with the equivalent dose of oral spray insulin. On both days, capillary blood glucose (Glucocard memory PC Menarini), venous blood glucose (by reference laboratory), plasma insulin and C-peptide levels (by RIA) were measured every 30 minutes for two hours and then every 60 minutes up until the fourth hour. **RESULTS:** Results of this study showed that there were no significant differences in blood glucose or insulin levels throughout the 4 hours after insulin administration between patients receiving the oral spray or the subcutaneous insulin formulation (BG at 4 hours: oral insulin = 101 mg/dl \pm 34 SD; subcutaneous insulin = 115.7 mg/dl \pm 50 SD). Insulin levels at 4 hours: oral insulin = 10.4 uU/ml \pm 3.3 SD; subcutaneous insulin = 13.6 uU/ml \pm 5.9 SD. No episodes of severe hypoglycaemia were observed.

CONCLUSION: Insulin administered via the buccal spray formula is effective in lowering blood glucose levels in patients with Type 1 diabetes similarly to insulin given by the subcutaneous route.

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REPLACEMENT OF SUBCUTANEOUS INJECTIONS WITH ORALIN IN TREATMENT OF DIABETES

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Background and Aims: Some diabetic patients refuse to take injections when oral agents fail. The alternative to this is the RapidMist, a novel Diabetes Management System. This system is based on the unique liquid aerosol formulation, which allows a precise insulin dose delivery in the form of fine aerosolized droplets directed in the mouth. This oral aerosol insulin formulation is rapidly absorbed through the buccal mucosal lining and in the oropharynx regions. The goal of this study was to evaluate the efficacy of the oral insulin (7 units, absorbable) versus s.c. injection (8 units) to control post-prandial glucose in type-2 diabetic patients after a standard meal challenge. **Materials and Methods:** In a single blind, randomized, crossover study, 11 type-2 diabetic patients received oral insulin (7 units, absorbable) via the RapidMist device or s.c. injection (8 units) followed by a 360 calorie Sustacal meal, 15 mins after the dose. Patients received both treatments in replicate dosing. **Results:** The table below shows serum glucose, insulin and C-peptide changes from the baseline after the dose of oral insulin or s.c. injection.

Time	Glucose mg/dl		Insulin pmol/l		C-peptide pmol/l	
mins	Injection	Oral	Injection	Oral	Injection	Oral
0	147	177	40	57	784	813
15	148	177	77	75	758	831
60	218	228	207	271	1464	1435
120	212	228	133	197	1495	1404
240	143	152	51	67	779	763

Conclusions: We conclude that the oral insulin absorption was comparable to s.c. injection as seen from the above data. This conclusion was supported by the statistical showing no significant differences between the treatments for glucose (p<0.314), insulin (p<0.810) and C-peptide (p<0.182). Oralin can be used safely in place of s.c. injections to treat diabetes.

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LONG-TERM EFFICACY OF ORAL INSULIN AND PIOGLITAZONE COMBINATION THERAPY FOR TREATMENT OF TYPE-2 DIABETES

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Background and Aims: Insulin injections possess the biggest challenge in the treatment of type-2 diabetes when oral agents fail and glucose levels are not controlled. Most patients refuse to take injections due to its invasive nature. An alternative to this is the introduction of needle free oral insulin (RapidMist) formulation. This system is based on the unique liquid aerosol formulation. This oral insulin formulation is rapidly absorbed through the buccal mucosal lining. The goal of the study was to evaluate the long-term (12 weeks or more) efficacy of the low dose of oral insulin (7units, absorbable) in combination with pioglitazone (30 mg) against placebo puffs + pioglitazone to improve the HbA1c in type-2 diabetic patients. **Materials and Methods:** In a double blind, randomized study, type-2 diabetic patients failing on oral agents received oral insulin (7units, absorbable) via the RapidMist device or placebo formulation in combination with pioglitazone tablets. The HbA1c levels were measured every 2-4 weeks. **Results:** The table below shows HbA1c profile at selected times.

Time (weeks)	0 weeks %HbA1c	2 weeks %HbA1c	4 weeks %HbA1c	8 weeks %HbA1c	12weeks %HbA1c
Pioglitazone+ Placebo puffs	9.6	10.0	10.1	10.3	10.6
Pioglitazone+ oral insulin	9	8.8	8.7	8.2	7.9

Conclusions: We conclude that the HbA1c levels significantly improved (1.1%, p<0.0001) in the group receiving oral insulin + pioglitazone when compared to the baseline, where HbA1c levels worsened with pioglitazone alone (up by 1%, p<0.1108). Thus, oral insulin can be used safely on a long-term basis without any adverse effect to treat diabetes in place of injections.

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Influences of oral insulin on carbohydrate, protein and lipid metabolism in the post weaning period in Balb/c mice.

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Background and Aims: Insulin is naturally present in human breast milk and has been demonstrated to possess trophic effects on the gut mucosa in suckling animals. These effects are localized to the gastrointestinal tract. The effects of oral insulin on mature animals have not been studied. We postulated that oral insulin supplementation would have a systemic metabolic effects on mature mice. **Materials and Methods:** Twenty Balb/c mice were put on oral insulin supplementation within drinking water (1 Unit of regular insulin/ml of drinking water) for 40 days after weaning. The control group received tap water. Both groups were fed on regular chow. The systemic effects tested included a lipid profile, prandial and fasting blood glucose levels, liver weight and protein content. Local effects tested included small intestinal weight and an histologic inspection on the intestine. **Results:** Total triglyceride level in the study group was significantly lower as compared with controls (130.40 mg/dl +/- 32.44 vs. 193.60 mg/dl +/- 48.47, $p < 0.02$). Total cholesterol level in the study group was significantly lower than levels of the control group (83.66 mg/dl +/- 5.75 vs. 93.38 mg/dl +/- 7.94, $p < 0.05$). Fasting glucose levels were significantly lower in the study group (mean + S.D. were 116.53 mg/dl +/- 10.78 compared with 143.92 mg/dl +/- 25.48 in controls, $p < 0.001$). Prandial glucose levels were measured on day 59 at 08.00am and levels were lower in the study group, similar to the fasting measurement (123.14 mg/dl +/- 12.25 vs. 145.16 mg/dl +/- 18.08, $p = 0.03$). Liver weight was elevated in the study group (0.75 gr +/- 0.11 vs. 0.67 gr +/- 0.09, $p = 0.048$) and so was the liver protein content (0.72 ng prot/mg tissue +/- 0.08 vs. 0.66 ng prot/mg tissue +/- 0.077, $p = 0.01$). **Conclusions:** Oral insulin supplementation had a significant influence on carbohydrate, protein and lipid metabolism. These effects have not been demonstrated before in mature animals. These observations may suggest some of the effects of insulin found in breast milk on breastfed infants beyond the neonatal period. Further studies are needed in this animal model to uncover the mechanism and explore the potential benefits of oral insulin supplementation to infant formulas.

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Clinical Application of Insulin Secretagogues (Sulphonylureas and Metiglinides)

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EFFICACY AND SAFETY OF NATEGLINIDE IN PATIENTS WITH MILD HYPERGLYCAEMIA

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Background and Aims: Diabetes patients with low fasting plasma glucose (FPG) levels may have significant prandial glucose excursions. Optimal treatment regimens when diet and exercise fail are not known, but nateglinide – which stimulates early insulin secretion with a fast-on, fast-off mode of action thereby lowering prandial glucose – may be clinically beneficial. A 24-week, multicentre, randomised, parallel-group, double-blind study was conducted to evaluate the efficacy and safety of nateglinide in patients with mild hyperglycaemia. **Materials and Methods:** Patients (n=675) with mean fasting plasma glucose (FPG) 7.0–8.3 mM were randomised to nateglinide 30 mg, 60 mg, 120 mg or placebo before 3 main meals (a.c.). Baseline mean values were: FPG, 7.6 mM; HbA_{1c}, 6.5% (range 4–9.1%); BMI, 29 kg/m² and diabetes duration, 3.6 years. **Results:** Mean (±SE) efficacy parameters (intent-to-treat population), as well as the plasma glucose (PG) and insulin response to a standard meal (assessed as area under the curve [AUC] from 0–4 hours) in a patient subset (n=127), are shown below:

Nateglinide/placebo	30 mg a.c.	60 mg a.c.	120 mg a.c.	Placebo a.c.
Δ HbA _{1c} (%)	-0.10 ± 0.05 [†]	-0.15 ± 0.04 [†]	-0.23 ± 0.05 [†]	0.16 ± 0.05 [*]
Δ FPG (mM)	0.08 ± 0.12 [†]	0.01 ± 0.11 [†]	-0.14 ± 0.12 [†]	0.59 ± 0.12 [*]
Δ PG AUC _{0-4h} (h*mM)	-1.97 ± 1.49 [†]	-3.63 ± 1.43 [†]	-3.95 ± 1.47 [†]	3.15 ± 1.33 [*]
Δ Insulin AUC _{0-4h} (h*μU/L)	36.95 ± 18.87 [†]	16.97 ± 17.76	46.78 ± 19.72 [†]	-12.60 ± 16.60

* $p < 0.05$ vs. baseline; [†] $p < 0.05$ vs. placebo

Confirmed hypoglycaemia (symptoms with PG ≤ 3.3 mM) occurred in 2.4, 4.0, 5.3 and 1.2% of patients on nateglinide 30, 60, 120 mg a.c. and placebo, respectively. The overall incidence of adverse events was similar in all treatment groups. Weight increased with both nateglinide (0.5–0.65 kg) and placebo (0.3 kg). There were no other clinically significant changes in the safety parameters. **Conclusions:** These results indicate that patients with early diabetes can be safely and effectively treated with agents such as nateglinide that target prandial hyperglycaemia.

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POLYMERIC NANOPARTICLES FOR ORAL DELIVERY OF INSULIN IN DIABETIC RATS.

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Background and aims: The most physiological and convenient route for the administration of insulin is the oral route. However, two main problems have to be overcome: (a) the degradation of insulin in the gastrointestinal tract by proteolytic enzymes and (b) the low intestinal absorption of insulin, a 51 amino acid peptide. Thus, we have associated insulin to nanoparticles (NP) composed of a biodegradable polymer, poly (epsilon caprolactone) (PCL) and a polycationic non biodegradable polymer of a polyacrylic nature, Eudragit[®] RS. The aim of this work was to analyse the biological effects of these NP given by gavage to diabetic rats. **Materials and Methods:** PCL/Eudragit[®] RS insulin loaded NP and empty NP (for control experiments) were formed by a water/oil/water solvent evaporation technique. They were administered by gavage in streptozotocin induced diabetic rats. Blood glucose and insulin levels were measured up to 24 hours after gavage. Oral glucose tolerance tests were performed 4 and 8 hours after gavage with NP. **Results:** 1) The size of insulin loaded NP was 358±12 nm while that of empty NP was 331±11 nm. 2) The yield of encapsulation of insulin was superior to 98%. 3) Insulin NP (100 U/kg body weight) administered by gavage to fasted diabetic rats, significantly reduced blood glucose levels by 36% ($p < 0.05$), 57% ($p < 0.01$), 57% ($p < 0.01$) and 14% ($p < 0.05$) respectively 4, 6, 8 and 24 hours thereafter, when compared to rats treated with empty NP. Insulinemia was increased at the same periods with a maximal effect 8 hours after gavage (+87%, $p < 0.05$). 4) Peroral insulin NP (100 U/kg b.w.) lowered the glycemic response to the oral glucose challenge (2 g glucose/kg b.w.) performed 4 hours later by 60% ($p < 0.01$), and by 38% ($p < 0.05$) during the 8 h test. Giving insulin alone perorally at the same concentration had no effect on glycemia. **Conclusion:** Insulin-loaded nanoparticles prepared with PCL and Eudragit[®] RS exert an antidiabetogenic effect when administered perorally in diabetic rats. These results may be explained by the mucoadhesive properties of the polycationic polymer and the release of encapsulated insulin by the biodegradation of poly(epsilon caprolactone), a non toxic polymer.

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NATEGLINIDE INDUCES EARLY-PHASE INSULIN IN PORTAL VEIN, WHICH CONTROLS THE POSTPRANDIAL STATE IN RATS

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Background and Aims: Postprandial hyperglycemia and hypertriglyceridemia are independent risk factors for cardiovascular disease in patients with type 2 diabetes. In the present study, we investigated the roles of early-phase insulin induced by nateglinide (NAT) on the postprandial glucose and lipid metabolism in normal and diabetic rats. **Materials and Methods:** NAT (50 mg/kg, p.o.) was administered to the rats with or without oral loading of glucose (1g/kg) or fat emulsion (2g/kg). The levels of portal insulin, blood glucose, plasma TG and gene expression in the liver were examined. **Results:** NAT remarkably increased the portal insulin levels with a peak at 5 min after oral administration. Blood glucose levels were lowered with the transient increase in glucokinase and decrease in glucose-6-phosphatase mRNA in the liver. NAT also lowered the peak glucose levels in glucose-loaded rats. In contrast, insulin (0.5U/kg) administered subcutaneously did not lower the peak glucose levels after glucose loading as NAT did, although it showed same hypoglycemic efficacy as NAT did in fasted rats. In addition, NAT also significantly suppressed the transient increase of plasma TG after fat loading (ΔAUC [0–4hr]: 15±69 vs. 501±112 mg · h/dl in Zucker fatty rats, 81±22 vs. 164±17 mg · h/dl in GK rats; n=10, $p < 0.01$). The decrease of TG was mainly at the origin and the pre β subfraction on agarose gel electrophoresis (including chylomicron and VLDL). **Conclusions:** These data indicate that the transient increase in portal insulin levels plays an important role in controlling postprandial state. Therefore, nateglinide may be effective for reducing both the postprandial hyperglycemia and TG-rich lipoproteins in patients with type 2 diabetes.

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NATEGLINIDE EXHIBITS GLUCOSE-SENSITIVE K_{ATP} CHANNEL-DEPENDENT AND INDEPENDENT INSULINOTROPIC ACTIONS

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Aims: To examine the K_{ATP} -channel-dependent and K_{ATP} -channel-independent insulin-releasing actions of nateglinide. **Materials and Methods:** Insulin release (mean \pm SEM) from clonal glucose-responsive BRIN-BD11 cells was measured during acute 20 min incubations ($n=6$). **Results:** Incubation with 50-200 μ mol/l nateglinide evoked 1.5-2.0-fold ($p<0.01$ - $p<0.001$) insulin-secretory responses at 1.1 mmol/l glucose (1.79 ± 0.11 ng/ 10^6 cells/20 min). The insulinotropic response to 200 μ mol/l nateglinide exhibited a glucose-dependent pattern with progressive 1.2-1.7-fold increases ($p<0.05$ - $p<0.001$) in insulin secretion from 5.6-30 mmol/l glucose. Under depolarizing conditions (16.7 mmol/l glucose plus 30 mmol/l KCl; 10.59 ± 0.43 ng/ 10^6 cells/20 min) 100-200 μ mol/l nateglinide evoked a 1.4-1.5-fold ($p<0.05$ - $p<0.001$) increase in insulin output. Nateglinide (200 μ mol/l) potentiated the insulinotropic effects of 5.6-30 mmol/l glucose by 1.3-1.4-fold ($p<0.001$) under depolarizing conditions. Prolonged (18 h) culture with 100 μ mol/l nateglinide did not alter basal insulin release, at 1.1 mmol/l glucose, but abolished the insulinotropic effects of 200 μ mol/l tolbutamide or glibenclamide. However, insulin-secretory responses to 200 μ mol/l nateglinide, 20 mmol/l leucine or 20 mmol/l arginine, although diminished were retained ($p<0.01$ - $p<0.001$). Interestingly, insulinotropic actions of 200 μ mol/l eforsan, 25 mmol/l forskolin or 10 nM PMA were unaffected by 18 h nateglinide culture. **Conclusions:** Nateglinide exhibits both K_{ATP} channel-dependent and K_{ATP} channel-independent insulin-secretory actions in BRIN-BD11 cells. The enhanced insulinotropic responses of this drug with increasing glucose concentration supports the antihyperglycaemic actions of nateglinide in type 2 diabetes.

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TARGETING POSTPRANDIAL HYPERGLYCAEMIA IN PATIENTS WITH TYPE 2 DIABETES: NATEGLINIDE VS ACARBOSE

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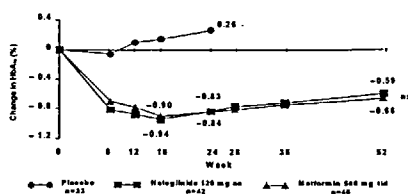
Background and Aims: The prandial glucose response depends on glucose absorption and suppression of endogenous glucose production. In patients with type 2 diabetes, absorption is normal but suppression of glucose production is diminished due to impaired early phase insulin secretory responses. The clinical utility of acarbose, which decreases glucose absorption, and nateglinide, which increases early phase insulin secretion, were compared in a double-blind, randomised 24-week trial in patients with type 2 diabetes. **Materials and Methods:** Patients with type 2 diabetes (179 men and women ≥ 30 years who had undergone diet and exercise treatment for at least 2 months before study entry) were randomised to receive either nateglinide (120 mg before 3 main meals, $n=87$) or acarbose (50 mg tid for 4 weeks followed by 100 mg tid, reduced again to 50 mg tid if necessary, $n=92$). Baseline HbA_{1c} values were comparable in the two treatment groups (7.75% and 7.87% for nateglinide and acarbose, respectively). A total of 144 patients completed the 24 weeks of treatment (70 on nateglinide; 74 on acarbose). **Results:** At the study endpoint, HbA_{1c} had been similarly reduced in both nateglinide and acarbose treatment groups, by 0.42% and 0.39%, respectively ($p=0.74$ for difference; intent-to-treat population and last observation carried forward). In patients completing the study, an HbA_{1c} level of $<7\%$ was achieved by 60% (42/70) of the patients receiving nateglinide, compared with 38% (28/74) receiving acarbose. Overall, adverse events were reported by 65% of patients receiving acarbose, compared with 49% of those receiving nateglinide. The incidence of gastrointestinal adverse events (mostly flatulence and diarrhoea) was greater in the acarbose-treated patients than in the group receiving nateglinide (42 vs 18 reports, respectively). Reports of symptoms suggestive of hypoglycaemia were recorded by 5.7% of patients (5/87) on nateglinide and 2.2% (2/92) on acarbose. **Conclusions:** Targeting post-prandial hyperglycaemia to control HbA_{1c} to levels below 7% is possible with suitable treatment. While nateglinide and acarbose demonstrated comparable efficacy, nateglinide may be preferable because of its superior side-effect profile, which may be a reflection of its more physiologically relevant mode of action.

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BOTH NATEGLINIDE AND METFORMIN SUSTAIN HbA_{1c} LOWERING OVER 52 WEEKS IN DRUG-NAIVE TYPE 2 DIABETES PATIENTS

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Background and Aims: Metformin is frequently used as initial therapy for type 2 diabetes due to a sustained reduction in HbA_{1c} with little or no hypoglycemia and weight gain, but it is inappropriate for use in some patients. Nateglinide is a new oral antidiabetic agent derived from the amino acid D-phenylalanine, which acts directly on the pancreatic β -cells to rapidly potentiate meal-induced insulin secretion thereby ameliorating postprandial glucose levels. Nateglinide is associated with <1 kg weight gain, $<0.3\%$ of patients dropping out of studies due to unassisted hypoglycemia and with no other treatment-emergent differences from placebo. This study compares the 1-year efficacy of nateglinide and metformin as initial therapy. **Materials and Methods:** Prospective, double-blind, placebo-controlled study in which drug-naïve patients (post hoc analysis) with ≥ 3 months' disease duration and mean baseline HbA_{1c} 8.1-8.3%, were randomized to placebo ($n=96$), nateglinide (120 mg before meals [ac], $n=97$) or metformin (500 mg tid, $n=97$) following a 4-week placebo run-in period ($n=33$, 42, 46 at 52 weeks, respectively). **Results:** No significant differences were apparent in HbA_{1c} between nateglinide and metformin over the 52-week study period (ANCOVA).



Conclusions: Nateglinide is as effective as metformin in initiating and sustaining improved glycemic control, indicating that nateglinide may be an appropriate alternative initial pharmacological therapy for type 2 diabetes.

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AN IMPROVEMENT IN INSULIN RESISTANCE AFTER THE ADMINISTRATION OF NATEGLINIDE

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Aims: Nateglinide is a newly administered active insulin secretagogue which stimulates the acute insulin response in patients with type 2 diabetes. We examined the mechanism and evaluated the effect of nateglinide on the glycemic control of types 2 diabetic patients being treated on an outpatient basis. **Materials and Methods:** A series of 28 type 2 diabetic patients visiting our outpatient clinic who agreed to take nateglinide were examined before and about 3 to 4 months after regularly taking of nateglinide (270 mg/day) including the standard meal test (460 Kcal). The BMI, IRI, blood glucose and HbA_{1c} levels were measured, and HOMA-R and HOMA- β were calculated according to the HOMA-model. **Results:** About 3 to 4 months later, nateglinide significantly decreased the mean area under the curve (AUC) for glucose during our standard meal test and a significant increase in the AUC for IRI was observed. The values of FBG, 1h-PG (1-hours post-prandial glucose), 2h-PG and HbA_{1c} improved from 165 ± 25 , 234 ± 39 , 229 ± 48 (mg/dl) and 7.5 ± 0.9 (%) to 148 ± 22 , 192 ± 35 , 178 ± 41 and 6.7 ± 0.7 before and after the above treatment regimen, respectively (MEAN \pm SD). When the patients were divided into two groups, including insulin resistant (IR: HOMA-R > 2 , $n=20$) and non-insulin resistant patients (NIR: HOMA-R ≤ 2 , $n=8$), the FBG only improved significantly in the IR whereas the 1h-PG, 2h-PG and HbA_{1c} levels improved in both groups. After the administration of nateglinide the HOMA-R level improved significantly from 4.0 ± 2.0 to 3.0 ± 1.6 ($p<0.02$) in the IR, but not in the NIR (from 1.2 ± 0.2 to 1.9 ± 0.9). Regarding insulin secretion, HOMA- β improved significantly only in the NIR from 16.4 ± 7.8 to 26.9 ± 9.9 ($p<0.02$), but not in the IR (from 32.3 ± 17.4 to 32.6 ± 19.0). **Conclusions:** Nateglinide was therefore found to be effective in controlling the blood glucose levels in type 2 diabetic patients by 1) improving the insulin resistance in IR patients by ameliorating glucose toxicity, and 2) improving the blood glucose levels in NIR patients by stimulating insulin secretion.

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EFFECTS OF GLIBENCLAMIDE ON HEMODYNAMIC AND METABOLIC STATUS AFTER MYOCARDIAL ISCHEMIA IN ISOLATED RAT HEARTS

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Background and Aims: It has been shown previously that the sulfonylurea derivative glibenclamide may improve post-ischemic cardiac functional recovery. Although K_{ATP} channel blockade is a possible explanation for this observation, an alternative mechanism may be operative. Therefore, we simultaneously recorded cardiac function and the intracellular concentration of ATP, PCr, Pi and pH before and after ischemia in the presence of glibenclamide or vehicle. **Materials and Methods:** ^{31}P magnetic resonance spectroscopy on erythrocyte-perfused, isolated working rat hearts was performed. 4 $\mu\text{mol.L}^{-1}$ glibenclamide or vehicle alone were tested (both $n=5$). The following protocol was used: 8 min performance assessment, 10 min drug treatment, 12 min global ischemia, 20 min reperfusion with drug treatment and 8 min functional recovery assessment. **Results:** Glibenclamide significantly decreased coronary blood flow as compared with vehicle: 59.5 ± 7.0 v. 94.3 ± 1.3 % ($p=0.008$). At the end of reperfusion, after ischemia, this vasoconstrictive effect of glibenclamide was even more pronounced (45.6 ± 5.4 v. 104.4 ± 5.9 %; $p<0.0001$). Further, glibenclamide significantly decreased the ischemia-induced cardiac functional loss as compared with vehicle (7.4 ± 1.3 v. 18.8 ± 3.3 %; $p=0.019$). During ischemia the intracellular acidosis was significantly attenuated in the presence of glibenclamide (final pH 6.75 ± 0.01 v. 6.43 ± 0.03 for vehicle, $p=0.005$). In addition, intracellular ATP tended to deplete more rapidly during ischemia in the presence of glibenclamide, however this did not reach statistical significance ($p=0.11$). **Conclusions:** Glibenclamide reduces coronary blood flow which may completely be attributed to vascular K_{ATP} channel blockade. Glibenclamide also reduces the myocardial functional loss after ischemia with preserving intracellular pH but not intracellular ATP levels during ischemia. This suggests that the beneficial response to glibenclamide is probably not the result of myocardial K_{ATP} channel blockade, but may be explained by inhibition of glycolysis.

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Beneficial effects of insulin vs. sulphonylurea treatment early in type 2 diabetes on stimulated C-peptide and metabolic control

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Background and aims: Traditionally, insulin treatment is instituted in type 2 diabetic patients only after life style measures and oral pharmacological agents, such as sulphonylureas, have failed to achieve acceptable metabolic control. Based on animal studies and studies in type 1 diabetes we hypothesized that a relative beta cell rest in type 2 diabetes would be advantageous for endogenous insulin secretion in comparison with continuous stimulation with sulphonylurea. The aim of the study was to investigate whether treatment with insulin started soon after diagnosis of type 2 diabetes is advantageous compared with glibenclamide treatment.

Materials and Methods: A Swedish multicenter 2-year open randomized clinical trial in 41 patients with ICA-negative non-insulin-dependent diabetes diagnosed 0-2 years before inclusion. Patients received either 2 daily injections of insulin Mixtard 30/70 or glibenclamide (3.5-10.5 mg daily). C-peptide-glucagon tests were performed yearly in duplicate after 3-4 days of temporary withdrawal of treatment.

Results: A questionnaire indicated no difference in well-being related to treatment. After one year the glucagon-stimulated C-peptide response was increased in the insulin-treated group by 0.11 nmol/l (95% CI -0.06 to 0.28, $p<0.065$), whereas it was decreased by 0.09 nmol/l (95% CI -0.27 to 0.08) in the glibenclamide group. The difference between groups was significant, $p<0.046$. After two years the respective effects were +0.08 for the insulin group and -0.02 for the glibenclamide group with no significant differences remaining between groups. HbA1c levels declined during the first year in both groups (-1.26% (95% CI -1.93 to -0.59) in the insulin, -1.04% (95% CI -1.58 to -0.50) in the glibenclamide group). At the end of the second year HbA1c was similar in the insulin group (+0.06) but rose in the glibenclamide group (0.71 (95% CI 0.15 to 1.26)). The difference in evolution of HbA1c during the second year were significant between groups, $p=0.013$.

Conclusions: Early insulin treatment vs. glibenclamide treatment in type 2 diabetes may prolong endogenous insulin secretion and promote better metabolic control.

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POSTPRANDIAL ACTIVATION OF COAGULATION IS INHIBITED BUT FIBRINOLYSIS IS NOT ALTERED BY ACUTE GLIMEPIRIDE ADMINISTRATION IN TYPE 2 DIABETIC SUBJECTS.

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Background and aims: Several abnormalities of coagulation and fibrinolysis are known to occur in diabetic subjects at the fasting state as well as postprandially. The aim of this study was to investigate the effect of the administration of a single dose of glimepiride on the postprandial activation of the coagulation and on fibrinolysis as compared to placebo. **Materials and Methods:** We designed a crossover randomised study. In the morning after an overnight fast, each diabetic subject ($n=12$, age 52-64 yr.) received a standard meal (600 Kcal, Carbohydrates 40% Lipids 50% Proteins 10%) preceded by one tablet of glimepiride (G, 2mg) or placebo (pl). The two tests were performed randomly, with an interval of 7 days. Blood samples were collected at baseline as well as 2 and 4 hours after the meal to measure blood glucose, insulin, and fibrinogen, d-dimers, F1+2 (prothrombin fragments 1+2), PAI-1 and tPA. Non parametric statistics were used for analysis. **Results:**

			Baseline	2 hours	4 hours
Fibrinogen	mg%	PI	198±59	220±57	198±59
		G	197±46	217±51	221±33
D-Dimers	µg/ml	PI	0,24±0,03	0,25±0,03	0,38±0,04*
		G	0,24±0,09	0,23±0,09	0,20±0,08*
F1+2	nM	PI	9,94±1,78	11,3±2,01	15,8±1,97*
		G	9,33±5,54	9,96±6,07	9,56±5,09*
PAI-1	ng/ml	PI	74,0±27,0	66,0±33,0	77,7±34,0
		G	58,9±40,0	60,8±45,7	33,7±23,4
t-PA	ng/ml	PI	13,2±3,1	11,4±1,3	11,6±2,1
		G	11,0±3,7	9,6±3,9	15,8±9,1

Placebo vs. Glimepiride * $p<0.05$

Glucose levels were lower and insulin levels higher after G administration. In these diabetic subjects postprandial levels of d-dimers, F1+2 (indexes of coagulation activation) increased significantly after the meal consumption. PAI-1 and tPA (indexes of fibrinolysis) did not change significantly postprandially. Acute administration of glimepiride reduced this activation 4 hours after meal consumption (Table). **Conclusions:** Acute administration of glimepiride, has an inhibitory effect on the postprandial activation of the coagulation but did not alter fibrinolysis.

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Improved Insulin Secretion and Sensitivity by Glimepiride in New Onset Type 2 Diabetes Mellitus.

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Background and Aims: The exact role of Glimepiride in improvement of glycemic control in Type 2 Diabetes Mellitus (DM) is unclear although its properties in enhancing glucose transporters and as an insulin secretagogue are documented. Therefore, insulin secretion and sensitivity were assessed in new onset Type 2 DM prior to (Pre) and after (Post) achieving desirable glycemic control (HbA_{1c} <7.0%) with Glimepiride treatment (Rx). **Methods:** Determination of plasma insulin (I) and glucose (G) after an overnight fast (F) and at every 15' for 2 hours after oral ingestion of glucose, 75 g (OGTT). Expression of 1) I (U/L) and G (mM/l) responses during OGTT as (a) cumulative response (CR) calculated by adding the differences between levels at times of determinations and F level; (b) difference between peak (P) and F levels (Δ). 2) Early insulin secretion as CRI over 30' and 60'. 3) Total insulin secretion during OGTT as $\Delta I/\Delta G$ and CRI/CRG (U/mM/l). 4) Insulin sensitivity as a product, $FI \times FG$ ($U \times mM/l$). **Results:** Pre-Rx FG, 14.0 ± 0.7 and PG, 26.0 ± 1.2 declined to 6.3 ± 0.2 and 15.3 ± 1.0 Post-Rx ($p<0.0001$ for both). Pre-Rx FI (16 ± 2) was markedly elevated and declined to 11 ± 1 post-Rx ($p<0.01$), but did not normalize (6 ± 1). However, early insulin secretion normalized (30', CRI, Pre-Rx, 6 ± 1 vs. 51 ± 7 Post Rx $p<0.001$ N, 52 ± 6 ; 60' CRI Pre-Rx 20 ± 3 , vs. 136 ± 16 Post-Rx, $p<0.001$; N, 111 ± 12). Moreover, total insulin secretion improved but did not normalize: $\Delta I/\Delta G$, Pre-Rx, 0.96 ± 0.2 vs. Post-Rx, 8.9 ± 1.8 , $p<0.001$ vs. N, 14.3 ± 2.1 $p<0.01$ and CRI/CRG, Pre-Rx 0.75 ± 0.2 vs. Post-Rx 7.4 ± 1.1 , $p<0.001$, vs. N, 19.8 ± 2.8 , $p<0.01$). Finally, pre-Rx insulin sensitivity index (241 ± 43) declined significantly post-Rx (65 ± 5) and normalized (29 ± 3) in 6 of 14 subjects with Type 2 DM. **Conclusion:** Glimepiride improves early and total insulin secretion as well as insulin sensitivity while achieving desirable glycemic control in new onset Type 2 Diabetes Mellitus.

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Clinical Hypoglycaemia (Type 1 and Type 2 Diabetes)

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Hypoglycaemia in patients with Type 2 diabetes in the UKPDS

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Background and Aims: The UKPDS demonstrated that a more intensive blood glucose control policy can reduce the risk of diabetes related complications in patients with type 2 diabetes. The DCCT similarly showed that an intensive blood glucose control policy was beneficial in type 1 diabetes. In the DCCT however, hypoglycaemia rates of 61 per 100 person years (py) were associated with the intensive glucose control policy, and the rate increased with decreasing levels of concurrent haemoglobin A1c (HbA1c). We have examined the self-reported hypoglycaemia rates seen in UKPDS patients and investigated the relationship to concurrent HbA1c and other factors over the first six years following diagnosis of diabetes. **Materials and Methods:** 2928 UKPDS patients randomised to an intensive glucose control policy following their dietary run-in period were included. Patients were asked specifically about hypoglycaemic episodes (graded from 1 to 4) at three monthly clinic visits and the grade of the most severe recorded. Episodes requiring remedial action by the patient or a third party (grade 2 or more) have been analysed. A binomial regression model was used to calculate absolute risk rates in relation to current HbA1c, concurrent therapy and sex. **Results:** Hypoglycaemia rates (95%CI) for intensively treated patients, allocated to and remaining on either insulin or sulphonylurea, were 3.3 per 100 py (0.1 to 45.4). In patients treated with basal insulin where hypoglycaemia rates increased from 3.2 per 100 py (1.8 to 5.5) to 7.7 per 100 py (6.3 to 9.5) over the same HbA1c range. Female patients reported higher hypoglycaemia rates than males (3.0 (2.6 to 3.6) vs 2.2 (2.0 to 2.4) per 100 py, $p < 0.0001$). **Conclusions:** Hypoglycaemia rates in patients with type 2 diabetes treated with a more intensive glucose control policy were higher in females, but overall, rates were substantially lower than those seen in intensively treated patients with type 1 diabetes, even when type 2 patients were treated with insulin. Concern about hypoglycaemia should not be a major limitation to implementing more intensive glucose control in people with type 2 diabetes.

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The effect of hypoglycaemia visual and auditory processing in man: a functional Magnetic Resonance Imaging Study

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Background and Aims: Hypoglycaemia with cognitive dysfunction is a feared side effect of insulin treatment. The performance of an action and the avoidance of danger in everyday life requires intact visual and auditory processing. We examined the effect of acute mild hypoglycaemia on visual and auditory processing in the brain using functional Magnetic Resonance Imaging.

Materials and Methods: Right handed volunteers were exposed to a visual and auditory stimulus during a euglycaemic- hypoglycaemic (5mmol/l and 2.4 mmol/l, $n=5$) and euglycaemia (5mmol/l, $n=5$) clamps. Brain activation was measured by functional magnetic resonance imaging (Blood Oxygen Dependent Imaging).

Results: There was a reduction in brain activation in the Primary Visual Cortex (Tallarch co ordinates: -9-81-7 voxel size2 & 40-72-7 voxel size1 & 14-94-2 voxel size1, and 3-72-7 voxel size4). The Inferior Temporal Lobe: -52-53-7 voxel size1 & 52-5-2 voxel size1) Increased activation was seen in the parastriate visual cortex: -38-75-7 voxel size2) All analyses run at a p value of 0.005. No global reduction in activation was identified, with most areas remaining unaffected.

Conclusions: We conclude that visual and auditory processing is preserved during hypoglycaemia, the brain areas involved demonstrating a very limited reduction in activation. The appreciation of these sensory modalities during hypoglycaemia is unlikely to be the limiting factor which results in cognitive dysfunction during hypoglycaemia.

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SEVERE HYPOGLYCAEMIA AND UNAWARENESS IN CHILDREN AND ADOLESCENTS TREATED AT SWEDISH GENERAL HOSPITALS

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Background and Aims: Treatment resources per Swedish diabetes patient have been gradually reduced during the 90ies along with the increased incidence of type 1 diabetes. The aim of this study was to investigate occurrence of self-perceived unawareness related to severe hypoglycaemia in type 1 diabetes children and adolescents treated at general hospitals in Sweden.

Materials and Methods: A geographic population of 333 intensively treated patients aged 3.9-20.1 years, (median 13.9), with duration 1.8-18.3 years (median 5.4), year mean HbA1c 4.7-13.4% (median 7.6, mean 7.8, SD 1.4, ref. range 3.7-5.0, method level 1.15% below DCCT), was asked to complete a questionnaire regarding severe hypoglycaemia during the last 12 months. Self-perceived unawareness was estimated using the previously used question "Can you feel when you are low in blood-glucose?" Patients responding "always" were classified as having normal awareness and those responding "usually", "occasionally" or "never" were classified as having impaired awareness.

Results: Data were obtained from 74% of patients. A mean of 2.5 physician visits per patient had taken place during last 12 months (range 1-5). A total of 81/247 (33%) reported having had one or several events of severe hypoglycaemia that required the assistance of another person during the preceding 12 months. Severe hypoglycaemia was reported from 62/152 patients reporting impaired awareness, but from 19/95 with normal awareness ($p=0.0002$).

Conclusions: A high proportion of this population with infrequent visits and suboptimal metabolic control reported impaired unawareness, and as expected their incidence of severe hypoglycaemia was higher. Structure of care need to be improved, aiming at good metabolic control and prevention of severe hypoglycaemia.

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Effect Of Rate Of Fall Of Blood Glucose On Cognitive Function In T1 DM Patients Aware and Unaware of Hypoglycemia

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Previous studies indicate that rate of blood glucose (BG) fall does not affect counterregulatory/symptom responses to hypoglycemia (H) in humans, but a faster fall of BG impairs cognitive function (CF) more than slow fall in T1 DM (Diabetes 49 (Suppl.1): A133, 2000). Aim of the present study was to assess whether a fast fall of BG differentially affects responses to H in aware vs unaware T1 DM patients. 12 T1 DM patients were classified as aware (N=6) or unaware (N=6) based on symptom responses to a previous stepped hypoglycemic clamp. All patients were studied on two occasions, during postprandial H plateau (BG 44 mg/dl for 30 min) with the hyperinsulinemic G clamp. In the slow-fall study, rate of BG fall was 0.6 mg/dl/min and H induced over 90 min, in the fast fall study rate of fall was 1.84 mg/dl/min and H induced in 30 min. Results (mean±SE): The rate of G fall did not affect counterregulatory hormone responses to H, except adrenaline and cortisol responses were greater in aware as compared to unaware patients in both studies ($p < 0.05$). As expected, symptom score was also higher in aware than unaware patients (12.2±2.6 vs 4.37±1.53, $p < 0.05$). CF (trail making A and B, PASAT, digit span backward, digit vigilance test, verbal memory test), expressed as z score, deteriorated at the end of H plateau vs baseline euglycemia in the slow-fall study to a similar extent both in aware and unaware T1 DM patients (z score, -1.59±0.30 vs -1.60±0.54, $p=NS$). In the fast-fall study, CF deteriorated more than in the slow-fall only in aware (-3.30±0.50, $p < 0.05$), but not in unaware (-1.89±0.40, $p=NS$). Single tests affected were Trail Making A (attention) (-1.56±0.80 vs -7.35±2.0, $p < 0.05$, unaware and aware respectively), Trail Making B (complex/divided attention) (-1.50±0.40 vs -3.04±0.49, $p < 0.05$), PASAT 2 sec. (information processing) (-0.31±0.32 vs -1.55±0.41, $p < 0.05$) and digit span backward (attention/memory retrieval) (-0.40±0.26 vs -1.98±0.32, $p < 0.05$). Thus, during H, not only the BG level achieved, but also the rate of BG fall exerts effects on CF in T1 DM. The observation that this occurs primarily in aware patients indicates that unaware patients are 'brain protected' against factors deteriorating CF such as fast fall of BG.

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REDUCTION IN SEVERE HYPOGLYCEMIA FOLLOWING USE OF CONTINUOUS GLUCOSE MONITORING: A PILOT STUDY.

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Background and Aims: Severe hypoglycemia is a major obstacle to achieving intensive treatment goals in type 1 diabetes. The limitations of self-monitored blood glucose (SMBG) to detect asymptomatic hypoglycemia, especially at night, contribute to the problem. In contrast, continuous glucose monitoring provides a dramatic increase in the amount of data available to the clinician attempting to minimize risk of severe hypoglycemia. This pilot study was conducted to determine if a reduction in the incidence of severe hypoglycemia could be achieved by using the Continuous Glucose Monitoring System (CGMS, MiniMed Inc.) in patients with a history of severe events.

Materials and Methods: 10 patients with a history of severe hypoglycemia (at least 1 event in the prior 3 months) wore the sensor for two evaluation periods. After each sensor wear, the glucose profiles were reviewed and therapy adjustments were made. The incidence of severe hypoglycemia was recorded at 3 months and 1-year follow-up.

Results: The 10 patients were 32 ± 16 years old with a 17 ± 10 year history of diabetes. Incidence of severe hypoglycemia decreased significantly after CGMS use for both the 3-month (total number of hypo events: 24 pre vs. 1 post, $p=0.007$) and 1-year (41 events pre vs. 8 events post, $p=0.007$) periods. No significant changes in either HbA1c (7.2 ± 0.9 vs. 7.2 ± 0.8 , $p=0.942$) nor insulin dose (28.5 ± 7.7 vs. 28.0 ± 5.2 , $p=0.794$) were observed over the same time periods.

Conclusions: These results demonstrate that use of the CGMS can have a marked and long lasting impact in reducing the risk of hypoglycemia even in well-controlled diabetic patients prone to severe hypoglycemia.

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Continuous glucose monitoring can help to decrease frequency of symptomatic hypoglycaemia in patients with type 1 diabetes treated with CSII

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Background and Aims: Availability of continuous glucose monitoring system (CGMS, MiniMed Inc.) offers the possibility to obtain 288 blood glucose readings a day through a 72 hour period. Previously published data showed better diabetes control when CGMS was used to identify appropriate therapy adjustment.

We wanted to determine if the use of CGMS in patients with type 1 diabetes treated with continuous subcutaneous insulin infusion will result in decreased number of symptomatic hypoglycaemias.

Materials and Methods: 9 patients (1 female and 8 men, age 38.4 ± 9.3 (mean \pm SEM)) with type 1 diabetes (duration 15.3 ± 7.38 , HbA1c $7.7 \pm 0.63\%$, 9.0 ± 3.8 symptomatic hypoglycaemias per month- during previous 4 months, total of 7 nocturnal episodes, no severe event) treated by CSII (duration 2.4 ± 1.46). Each patient wore CGMS for 3 working days during his normal activity. Important events including insulin, exercise, meals and hypoglycemic episodes were self-recorded. After CGMS use continuous glucose profiles were reviewed to identify changes in therapy. Therapy adjustment included changes to insulin dosage, diet and treatment of high and low blood glucose values. Symptomatic hypoglycaemic episodes were recorded for next 8 weeks and HbA1c values were obtained in week 8.

Results: We found a decrease in number of symptomatic hypoglycaemias per month: 5.11 ± 1.76 (no nocturnal or severe event) vs. 9.0 ± 3.8 in previous period (7 nocturnal, no severe episode), $p < 0.05$. We also found a decrease in HbA1c: $6.77 \pm 0.6\%$ in week 8 v.s. $7.7 \pm 0.63\%$ at baseline, $p < 0.09$.

Conclusions: Continuous glucose sensing provides meaningful data for individuals to personalize their pump insulin regimen to reduce risk of symptomatic hypoglycaemia and to obtain better diabetes control.

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Patient Treatment Satisfaction is Not Affected by Frequency of Hypoglycaemia in Type 1 Diabetes: Investigation with Continuous Subcutaneous Blood Glucose Measurement

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Background and Aims: To investigate relationships among reported frequency of symptomatic hypoglycemia, low blood glucose (BG) values in self-monitoring/in continuous subcutaneous glucose measurement and treatment satisfaction in type 1 diabetes. **Materials and Methods:** Prospective pilot study in sixteen type 1 diabetic patients (age: 44 ± 9 SD, diabetes duration: 18 ± 10 years) using functional insulin treatment (FIT: basal, prandial and correctional dosages). Observation period was 181 \pm 11 days. Patients reported 27 (median, 6-98) symptomatic hypoglycemic episodes, which have always been accompanied by a BG self-measurement and documentation of symptoms. Self-monitoring (6.2 ± 1.9 /day) was performed with Accutrend Sensor Complete® glucometer. HbA1c ($6.8 \pm 0.7\%$), ref 4.2 - 6.0% , and treatment satisfaction have been investigated repeatedly. The follow-up included subcutaneous measurement via glucose sensor (CGMS Minimed®) for 73 ± 28 hrs. **Results:** In self BG monitoring, 1.3% values were below 40, 2.9% below 50 and 3.9% below 60 mg/dl, whereas 22.6% of all values were above 200 mg/dl. HbA1c correlated (0.75 , $p=0.001$) with perceived number of symptomatic hypoglycemia and with number of BG readings above 200 mg/dl. Frequency of BG readings above 200 mg/dl correlated (0.53 , $p=0.03$) with that of symptomatic hypoglycemia. CGMS confirmed that subjective perception of hypoglycemia diverges from objectively recorded low BG values. Ten percent ($10 \pm 8\%$, range 0.3 - 23%) of the sensor recording time displayed BG values below 40 mg/dl. Treatment satisfaction assessed with DTSQ questionnaires showed no relationship neither with perceived (-0.3 , $p=0.9$) nor recorded hypoglycemia, but was significantly inversely related to frequency of high BG readings (-0.5 , $p=0.05$). **Conclusions:** A higher HbA1c and frequency of hyperglycemia is associated with more frequently perceived hypoglycemia. No relationship exists between perceived hypoglycemia frequency and hypoglycemic values in self BG monitoring, as well as in CGMS. In type 1 diabetic patients with FIT, glycemic control assessed by HbA1c and frequency of perceived and/or recorded hypoglycemia does not influence patients treatment satisfaction.

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Risky Detection Delay of Fast Blood Glucose Changes by Glucose Monitoring at the Arm.

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Background and Aims: Alternate site glucose monitoring of capillary blood glucose (BG) is an attractive new option in addition to measurements at the finger. But patients with diabetes reported discrepancies between clinical symptoms of hypoglycaemia and normoglycaemic values at the arm during self-monitoring. As technical and handling problems could be excluded, we examined the question whether fast BG changes over a large range result in clinically relevant BG differences between arm and finger and whether local rubbing at the arm could influence such differences. **Materials and Methods:** Capillary BG samples were taken from the fingertip and the forearm of 23 diabetic patients on intensified insulin treatment (diabetes type 1/2: 18/5, age: 18-62 years, diabetes duration: 0.1 - 28 years) using the following meters: FreeStyle (TheraSense, USA), Soft-Sense (MediSense/Abbott, USA) and OneTouch Ultra (Lifescan, USA) according to the following protocol: After an overnight fast the usual pre-breakfast insulin was omitted and the breakfast was replaced by an oral glucose load (equivalent to 75g glucose) (Dextro O.G.T., Roche, Germany) to achieve BG values of 300-400 mg/dl. Then the patients' usual short acting insulin was given intravenously at an individual dose (6 - 40 IU/injection). The capillary BG was followed every 15 min until either steady state or hypoglycaemia (<60 mg/dl) was reached. **Results:** There have not been any device specific differences. At the fasting steady state the BG values at the finger (mean \pm SD: 129 ± 33 mg/dl) and at the arm (121 ± 32 mg/dl) were similar for all patients. The BG increase at the arm (150 ± 33 mg/dl) was smaller than at the finger (205 ± 36 mg/dl within 86 ± 22 min) ($p < 0.01$). During fast BG increase the arm BG was lagging behind the finger BG by 30 minutes (range: 6-91) at 250 mg/dl. During fast BG decrease [3.1 ± 1.0 (mg/dl)/minute] the arm BG was lagging behind the finger BG by 37 minutes (range: 19-67) at 100 mg/dl. At the first hypoglycaemic fingertip value (44 - 63 mg/dl) the arm BG differed by a mean of $+62$ mg/dl (range: $+26$ - $+106$) ($p < 0.01$). For the 3 patients with hypoglycaemic unawareness, the first asymptomatic hypoglycaemic values at the finger ($44/51/53$ mg/dl) were accompanied by normoglycaemic values at the arm ($106/142/159$ mg/dl). Rubbing decreased the observed differences on average by half but with a large intra- and interindividual variability. **Conclusions:** To avoid risky delays of hyper- and hypoglycaemia detection BG monitoring at the arm should be strictly limited to situations in which ongoing fast BG changes can be excluded.

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Sustained hormonal response to hypoglycemia in type-1 diabetes is associated with residual C-peptide

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Background and Aims: We had reported before on complete lack of severe hypoglycemia in a cohort of adult patients with newly diagnosed type-1 diabetes (Linn 1996, 1999). We studied differences in the hormonal response to hypoglycemia in type-1 diabetic patients with residual C-peptide mobilised by a stimulus compared to patients with residual C-peptide not responsive to this stimulus.

Materials and Methods: Hypoglycemic clamps (blood glucose 2.6 mmol/l at 135-150 min) were performed in non-diabetic controls (n=6), type-1 diabetic patients with C-peptide response to 1 mg i.v. glucagon (responders, R, n=6) and patients (n=6) not responding (NR) to glucagon. Subjects were matched for age and weight, patients also for diabetes duration. Mean stimulated C-peptides were control: 1.3 ± 0.5 , R: 1.2 ± 0.8 , and NR: 0.2 ± 0.1 mmol/l. Cortisol, glucagon, epinephrine, norepinephrine, and growth hormone (GH) were measured every 15 min. Autonomic and neuroglycopenic symptoms were assessed at eu- and hypoglycemia. ANOVA for repeated measures and multiple linear regression was carried out using SPSS.

Results: Patients had diabetes for 1.1 ± 0.7 years, GHb 7.3 ± 1.6 %, and daily insulin dose 0.45 ± 0.1 U/kg. These parameters were not different between R and NR. Mean glucagon response was significantly higher in R compared to NR (112 ± 13 vs. 91 ± 3 pg/ml, $p < 0.001$). Mean GH secretion in R was lower (9.4 ± 7.1 vs. 15.2 ± 9 ng/ml, $p < 0.01$). Neither autonomic nor neuroglycopenic symptom score was significantly different. Glucagon release subsequent to hypoglycemia was positively related to stimulated C-peptide. Interestingly, GH response was decreased in the R-group.

Conclusions: We cannot decide whether these effects are directly dependent on C-peptide action or other mechanisms associated with persisting insulin reserve of the endocrine pancreas.

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RECOVERY OF GLUCAGON RESPONSES TO HYPOGLYCEMIA OCCURRING IN THE POST-PRANDIAL STATE IN T1 DM

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Glucagon (IRG) responses to hypoglycemia (H) seem irreversibly blunted/absent in the natural history of T1 DM with progressive loss of pancreatic B-cell function. If it were possible to recover IRG responses to H, safety of intensive insulin therapy in T1DM would increase. Aim of these studies was to assess whether IRG responses to H recover in the postprandial (PP) vs fasting (F) state. 5 patients with T1DM (age 31 ± 3 years, diabetes duration 17 ± 3 , C-peptide < 0.02 nmol/l, HbA_{1c} 7.1 ± 0.2) and 5 nondiabetics were studied on 2 random occasions, in F and PP (mixed meal 447 Kcal, 46% CHO, 22% protein, 32% lipids) state. On both occasions, H was induced by i.v. insulin (2 mU/kg/min for 205 min) + variable glucose infusion to clamp the fall of plasma glucose (PG) from baseline euglycemia (E) to 44 mg/dl in 90 min, to maintain the plateau PG of 44 mg/dl for 30 min, and to recover PG to E in 70 min. Results of IRG (pg/ml):

	Basal Euglycemia	Hypoglycemia	Recovery Euglycemia
T1 DM			
Fasting	110 ± 13	118 ± 14	102 ± 11
Postprandial	122 ± 19	$195 \pm 45^{**}$	127 ± 41
Nondiabetics			
Fasting	195 ± 30	215 ± 36	154 ± 29
Postprandial	212 ± 26	$400 \pm 42^{**}$	265 ± 35

(* $p < 0.05$ vs basal and recovery euglycemia; ** $p < 0.05$ vs fasting hypoglycemia)

Adrenaline responses to H were increased by meal in nondiabetics (~50%) and T1 DM (~15%). Thus, in both nondiabetics and T1 DM, IRG responses to H increase in PP state. This is likely the result of meal aminoacid (AA) stimulation of pancreatic A-cell although a contribution by increased adrenaline on A-cell cannot be excluded. In nondiabetics, PP state exaggerates IRG responses which do occur in F state. In T1 DM, PP state recovers the missing responses in the F state. Because in T1 DM in the PP state IRG responses increase in H, but decrease to baseline after recovery of E, whereas AA remain increased in both H and E, it is concluded that AA do not stimulate pharmacologically the A-cell, but exert a permissive role for responses of IRG specifically during H.

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Plasma glucose disappearance rate and restoration of euglycemia are not affected by repeated insulin-induced hypoglycemia in normal volunteers

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Background and Aims: Hypoglycemia represents a major acute complication of type 1 diabetes mellitus and its therapy, contributing to both its morbidity and mortality.

Aim of the study was to evaluate in eight healthy male volunteers the effect of rapid impulsive, sequential perturbations of the glucose-insulin system produced by I.V. boluses of rapid-acting insulin (0.025, 0.05 and 0.1 uIU/kgbw insulin received in randomly sequence).

Materials and Methods: Each subject (mean age 25 ± 3 yrs; BMI 22.8 ± 2.0 kg/m²) received the three doses of insulin in three successive periods, separated by suitable recovery times. Glycemia was analyzed at -10, -5, and 0 min, every 1 min for 30 minutes, and every 5 minutes thereafter, until plasma glucose level returned to 80% of the basal value; insulin at -10, -5, 0, and 5 min, and every 15 minutes thereafter for the entire experiment. Counterregulatory hormone peaks (epinephrine, norepinephrine, cortisol, GH, and glucagon) were also measured. For each period, the first decreasing portion of the glucose curve, from period baseline to the period Nadir, was fitted by ordinary least squares to a monoexponential decay giving the parameter KITT. The parameter T80 was estimated by linear interpolation of neighboring concentration points and expresses the time needed for recovery from Nadir to 80% period baseline.

Results: KITT was not significantly affected by the amount of insulin injected nor by the period. Plasma glucose Nadir significantly ($P = 0.022$) decreased with higher administered insulin doses. Higher doses of injected insulin resulted in longer T80 values ($P = 0.002$). The peak height of counterregulatory hormones was not significantly dependent either on the period examined or on the dose of insulin administered. Epinephrine peak levels were negatively correlated with the Nadir level ($R = -0.429$, $P = 0.037$), and positively correlated with T80 ($R = 0.484$, $P = 0.017$).

Conclusions: The present study indicates that plasma clearance of glucose after different I.V. doses of insulin is a constant for a given subject, while the Nadir is dependent on the amount of insulin administered. Furthermore, the recovery of plasma glucose concentration to at least 80% of the basal value is always reached after 600 minutes of fasting and three doses of insulin, indicating that at least in healthy subjects there is no exhaustion of counterregulatory hormone response.

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DOES LONG-TERM THEOPHYLLINE TREATMENT IMPROVE HYPOGLYCEMIA UNAWARENESS IN TYPE 1 DIABETES?

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Background and Aims: In type 1 diabetic patients, single-dose administration of the adenosine receptor antagonists theophylline or caffeine has been shown to improve metabolic responses to and awareness of hypoglycemia. Long-term use of these agents has been associated with development of tolerance, restricting its potential efficacy in treating hypoglycemia unawareness. We therefore investigated the effect of long-term theophylline on responses to hypoglycemia in type 1 diabetic patients with hypoglycemia unawareness.

Materials and Methods: Twelve patients participated in a randomized placebo-controlled double-blind cross-over study. Slow-release tablets containing either 250 mg theophylline or placebo were used twice daily for 2 weeks. Thereafter, hyperinsulinemic (60 mU/m²/min) hypoglycemic (4.8-3.4-2.4 mM) glucose clamps were performed. During the clamps, both objective (glucose infusion rates [GIR], hemodynamics, and sweat detection using dew point electrode) and subjective (symptoms using checklists) parameters were recorded.

Results: During theophylline, hemodynamic responses to hypoglycemia were more pronounced for heart rate ($+10 \pm 4$ vs $+24 \pm 2$ bpm, $P < 0.05$), pulse pressure ($+3 \pm 2$ vs -1 ± 2 mmHg, $P < 0.05$), and pressure-rate product ($+708 \pm 538$ vs -371 ± 273 bpm*mmHg, $P < 0.05$) compared to placebo. With theophylline, sweating responses tended to occur at higher glucose levels (2.52 ± 0.12 vs 2.28 ± 0.08 mM, $P = 0.08$). Finally, GIR at hypoglycemic nadir was lower during theophylline as compared with placebo (2.4 ± 0.5 vs 3.0 ± 0.3 mg/kg/min, $P < 0.05$), indicating augmented counterregulatory hormone release. In contrast, subjective symptom scores were not significantly altered by theophylline.

Conclusions: During long-term treatment with theophylline, we still observed an augmented counterregulatory response to hypoglycemia, which may help recovery from and perception of hypoglycemia. The negative results on subjective symptoms indicate that the benefit of theophylline treatment may be further improved by awareness training focussed on the hemodynamic and sweating responses to hypoglycemia.

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Clinical Effects of Thiazolidinediones in Type 2 Diabetes

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Patient characteristics and drug treatment patterns associated with use of newer thiazolidinediones.

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Background and Aims: One of the newest drug classes used in the treatment of type 2 diabetes is the thiazolidinediones (TZDs). The first TZD (troglitazone) was approved in the U.S. in 1997 but removed from the market in 2000. Two newer TZDs (rosiglitazone and pioglitazone) were approved in the U.S. mid-year 1999. This study examines the patient characteristics and prior drug treatment patterns associated with receiving a prescription for a newer TZD after U.S. approval. **Materials and Methods:** Health plan enrollment data, medical claims, and pharmacy claims from the MarketScan databases were used to construct a sample of patients receiving drug therapy for diabetes. The sample included 42,531 type 2 diabetes patients who received at least one prescription for an anti-diabetic drug and were continuously enrolled between 1/1/1998 and 5/24/1999 (the study pre-period). Of these, 21,427 also had claims and enrollment data from 5/25/1999 through 6/30/2000 (the study follow-up period). We excluded an additional 220 patients who initiated troglitazone during the follow-up period. **Results:** The final sample included 21,207 patients; 6,302 of these received a newer TZD during the follow-up period. Mean age of patients was 63.5 years (SD=11.5); 11,443 patients (54.0%) were male. Logistic regression results indicated that TZD use was significantly higher ($p<0.05$) among patients with pre-period use of troglitazone (odds ratio [OR]=9.3), sulfonylureas (OR=1.6), alpha-glucosidase inhibitors (OR=1.5), or metiglinide analogues (OR=2.0). It was also significantly higher for patients with more pre-period diabetes drug use (OR=1.03 per 30 days), patients without diabetes complications (OR=1.1), and those with prior coronary artery disease (OR=1.1). TZD use was significantly lower among patients with pre-period use of insulin (OR=0.6) and biguanides (OR=0.6). Age has an inverted u-shape relationship with TZD use. The odd-ratios associated with age and aged squared are 1.5 (per 10 years of age) and 0.96 (per 10 years of age, squared), respectively. TZD use was not associated with patient sex or with insurance type. **Conclusions:** This study provides an early look at the characteristics of patients who are being prescribed newer TZDs. TZD use is associated with prior diabetes drug treatment patterns, diabetes severity, and comorbidities. These factors may confound observed differences in outcomes between patients using TZDs and other anti-diabetic drugs.

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Muscle fat content predicts clinical efficacy of pioglitazone in type 2 diabetic patients

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Background and Aims: We have previously reported that computer tomography (CT) density of skeletal muscle strongly predicts the clinical efficacy of troglitazone in type 2 diabetic patients, suggesting that muscle fat plays an important role in the mechanism of thiazolidinedione derivatives on glucose metabolism. To confirm this observation, we assessed how regional body fat affected the improvement of glycemic control induced by pioglitazone in type 2 diabetic patients.

Materials and Methods: Both visceral and subcutaneous abdominal fats as well as muscle fat were determined with CT scanning in 20 type 2 diabetic patients (9 men and 11 women, age: 60.0±2.4, BMI: 25.6±0.7Kg/m²) who were poorly controlled with sulfonylurea or diet (HbA1c: 8.3±0.3%). Muscle fat content was assessed from the density values of cross-sectional CT in hip and thigh muscle. Pioglitazone (30 mg/day) was supplemented for up to 6 months, and then we evaluated how each regional fat content affected improvement of HbA1c (altered % of initial values).

Results: Supplementation of pioglitazone decreased HbA1c to 80.7±2.1% of the initial values, but there was a wide dispersion from 94.1 to 63.6%. Simple linear correlation analysis revealed that both initial thigh and hip muscle CT densities and initial HbA1c levels were significantly correlated to the improvement of HbA1c after the supplementation of pioglitazone (thigh: $R=0.64$, $p<0.005$, hip: $R=0.53$, $p<0.05$, HbA1c: $R=0.74$, $p<0.001$). However, none of age, BMI, initial fasting IRI, abdominal visceral or subcutaneous fat area were related to the improvement of HbA1c. In stepwise multiple regression analysis using variables, age, BMI, fasting IRI, initial HbA1c, abdominal visceral and subcutaneous fat area, and muscle CT density, three variables entered the regression equation for the improvement of HbA1c: the first was initial HbA1c ($R^2=0.48$), the second was thigh muscle CT density ($R^2=0.59$), and the third was hip muscle CT density ($R^2=0.72$) showing that these three factors accounted for 72% of variances in the improvement of HbA1c.

Conclusions: We conclude that muscle fat content strongly predicts the clinical efficacy of pioglitazone in type 2 diabetic patients. We thereby suggest that muscle fat content is important in the mechanism of thiazolidinedione derivatives.

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Clinical Trial Experience of Risk for Liver Enzyme Elevation with Concurrent Pioglitazone and HMG-CoA Reductase Inhibitors.

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Background and Aims: The relationship between use of thiazolidinediones (TZD) and liver enzyme elevation is of clinical importance. Troglitazone, the first TZD marketed, was removed from use due to liver toxicity. While no predictive relationship between liver enzyme (LFT) elevation and TZD-related toxicity was defined, routine LFT monitoring is recommended for other TZDs in hopes of early detection of any serious liver toxicity. Patients with Type 2 diabetes are at greater risk for a variety of liver disorders and are routinely exposed to multiple pharmacologic agents. In a cohort of patients treated with troglitazone in combination with an HMG-CoA reductase inhibitor (statin), we recently described a 9-fold increased risk versus troglitazone alone, for LFT elevations greater than 3x the upper limit of normal (ULN). Thus it appears that concurrent statin and TZD use may represent a potential risk for development of significant LFT elevations.

Materials and Methods: Liver enzyme activity (ALT & AST) in patients with Type 2 diabetes who participated in 6 clinical trials using pioglitazone (PIO) was compiled. Demographic and concurrent lipid lowering drug therapies were tabulated. PIO doses used in these trials ranged from 7.5 to 45 mg per day. Complete data was available for 1519/1524 patients (676 female, 843 male). LFT were measured at baseline and 7.3 ± 1.7 times during PIO exposure in each patient. Since PIO is approved for clinical use in doses ranging from 15 to 45 mg per day, patients with PIO exposures less than 15 mg per day and those with LFT elevations greater than ULN at baseline were excluded. Data are mean±SD with significance per t-test or Chi-squared test if appropriate.

Results: Initially, 1.5x ULN elevations in ALT or AST occurred in 128/1519 (8.4%) with 3x ULN elevations in 6/1519 (0.4%). The final cohort had 1.5x ULN LFT elevations in 27/1223 (2.2%) of patients and 3x ULN elevations in 2/1223 (0.2%). PIO exposure was 113±41 days with no difference ($p>0.2$) between statin user and nonuser groups. Gender mix was similar ($p>0.6$) between statin users (80 F, 108 M) and nonusers (457 F, 578 M). BMI was no different ($p>0.1$), statin users 31.4 ± 5.1 kg/m², nonusers 32.0 ± 5.5 kg/m². No difference ($p=0.09$) in 1.5x ULN elevations was detected between statin and non-statin users. There were no LFT elevations 3x ULN noted in the concurrent statin group.

Conclusions: Contrary to findings with troglitazone and statin use, no increase risk of LFT elevation was noted at approved PIO doses with concurrent statin use when those with abnormal baseline LFT are excluded from analysis.

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Pioglitazone reduces intra-abdominal fat

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Background and Aims: Type 2 diabetes and the metabolic syndrome are underpinned by insulin resistance and characterised by obesity and central fat distribution. However increased insulin sensitivity promotes lipogenesis. This study tests the hypothesis that pioglitazone, an insulin sensitising agent, might have adverse effects on body fat.

Materials and Methods: A randomised placebo-controlled trial of pioglitazone 45 mg/day for 18 weeks in type 2 diabetic patients treated only with diet aimed at weight maintenance.

Results: In controls (n=12) there was no change in body weight (0.0, CI -1.1 to 1.0 kg) but patients treated with pioglitazone (n=13) gained 2.5 (CI 0.7 to 4.2) kg, of which 2.2 (CI 0.6 to 3.9) kg was fat (deuterium dilution method). Intra-abdominal fat mass, however, showed a significant reduction of 0.12 (CI -0.19 to -0.06) kg compared to controls (Magnetic Resonance Imaging). There were significant increases in triceps and biceps skinfold thicknesses and in hip circumference amongst patients treated with pioglitazone compared to controls, but no significant differences in waist circumference or waist/hip ratio. These body composition changes were associated with significantly reduced HbA1c (-1.5%) and fasting glucose (-2.4 mmol/l) compared to placebo. Reductions in triglycerides (-1.22 mmol/l) and LDL cholesterol (-1.8 mmol/l) on pioglitazone were not significantly different from placebo.

Conclusions: Although pioglitazone treatment is associated with whole-body lipogenesis, intra-abdominal fat is reduced, in keeping with its central role in the metabolic syndrome, and an effect of pioglitazone on PPAR gamma principally in subcutaneous adipose tissue. Future studies should combine glitazone treatment with a weight loss programme.

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TYPE 2 DIABETES MELLITUS DISEASE PROGRESSION AND TREATMENT WITH PIOGLITAZONE (ACTOS®): A POPULATION MODEL APPROACH.

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Background and Aims: Treatment efficacy in patients with type 2 diabetes mellitus is usually evaluated by analysis of variance with "last observation carried forward" approaches that cannot discriminate between inter- and intra-individual variability of observations. The present study contains a population model approach for evaluation of glycaemic control with a mechanism-based model. **Materials and methods:** Data from a 6-month randomized, placebo-controlled monotherapy study (7.5–45 mg/day) were evaluated using a non-linear mixed effect model. Glycaemic control, characterized by fasting blood glucose (FBG) and HbA_{1c} levels, was described as zero-order FBG influx and first order decrease with time-dependent rate, leading to a pathological state. HbA_{1c} levels were described as a delayed function of FBG. Treatment effects were expressed by counteracting the decrease of the glucose removal rate up to 100%, depending on the patient's observations. **Results:** The population model gave an adequate description of the data. After accounting for inter-individual variance, the residual error for the model fit of HbA_{1c} observations was smaller than for the FBG observations (0.1 vs. 0.6 %). A delayed response of HbA_{1c} to FBG changes was observed, with a half-life of 2.5 weeks. In addition, simulations showed that a number of 50 patients per treatment group is sufficient for identification of the significance ($P < 0.05$) between placebo and the lowest active drug treatment group. **Conclusions:** A good description of glycaemic control before and during treatment of type 2 diabetes mellitus was achieved with the present approach. The model brings observations for FBG and HbA_{1c} under a single, common denominator with a high precision for both types of observations. The combination of population statistics and mechanism-based modelling increases the sensitivity for evaluation of the significance of different treatment regimes.

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PIOGLITAZONE DECREASES INSULIN RESISTANCE IN PATIENTS WITH TYPE 2 DIABETES ON METFORMIN

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Background and Aims: Pioglitazone (PIO) is a thiazolidinedione that targets insulin resistance and lowers plasma glucose and insulin levels in patients with type 2 diabetes mellitus (T2DM). Recently, QUICKI ($1/[\log(\text{fasting insulin} \times \text{fasting glucose})]$), was shown to correlate well with the hyperinsulinemic euglycemic clamp method. We studied the effect of pioglitazone therapy on insulin sensitivity, by the QUICKI method, in patients with T2DM currently treated with metformin (MET) and not in good glycemic control ($\text{HbA}_{1c} \geq 8.0\%$). **Materials and Methods:** The QUICKIs of patients with T2DM who participated in a multicenter, double-blind clinical trial were calculated and compared for treatment effects. After a 6-week run-in period, patients were randomized to placebo in combination with MET ($n=148$) or PIO 30 mg QD in combination with MET ($n=157$). Patients remained on the assigned treatment for 16 weeks. Fasting blood samples were obtained for HbA_{1c}, plasma glucose, and insulin. An analysis of covariance (with treatment, center, and baseline included as a covariate) of percent change (%Δ) from baseline (last observation carried forward) in QUICKI was performed. The least-squares mean %Δ for the PIO+MET group was compared with that of the Placebo+MET group.

Results: The results for QUICKI are shown:

	Baseline QUICKI	% Δ	SEM	P-value vs Baseline	P-value vs placebo
Placebo + MET	0.293	0.3%	0.59	P=0.57	
PIO + MET	0.295	5.4%	0.57	P=0.0001	P=0.0001

In addition, PIO lowered HbA_{1c} by 0.83% points and FPG by 2.36 mmol/L, compared with placebo.

Conclusions: In this study QUICKI proved to be capable of detecting differences in insulin sensitivity between pioglitazone and placebo in patients with T2DM currently treated with MET. Based on the QUICKI methodology, pioglitazone significantly improves insulin resistance, compared with both baseline and placebo.

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PIOGLITAZONE REDUCES ATHEROGENIC INDEX OF PLASMA IN PATIENTS WITH TYPE 2 DIABETES ON SULFONYLUREA

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Background and Aims: The Atherogenic Index of Plasma (AIP) is $\log(\text{Tg}/\text{HDL-C})$ and correlates inversely with LDL particle size ($r = -0.78$). Cohorts with low risk for coronary heart disease (CHD) have below zero AIP values and those with high risk have positive values. People with type 2 diabetes mellitus (T2DM), at high risk for CHD, have higher AIPs than their matched controls. We studied the effect of pioglitazone (PIO), a thiazolidinedione, on AIP in patients with T2DM currently treated with sulphonylurea (SU) and not in good glycemic control ($\text{HbA}_{1c} \geq 8.0\%$). **Materials and Methods:** The AIPs of T2DM patients who participated in a multicenter, double-blind clinical trial were calculated and compared for treatment effects. After a 6-week run-in period, patients were randomized to placebo ($n=175$), PIO 15 mg QD ($n=171$), or PIO 30 mg QD ($n=179$); they remained on the assigned treatment for the next 16 weeks. Fasting blood samples were obtained for HbA_{1c}, plasma cholesterol (total-C, LDL-C, and HDL-C), Tg, glucose, and insulin. The least-squares mean change (Δ) in lipids, HbA_{1c}, and AIP from baseline (last observation carried forward) for each group was obtained from analysis of covariance model with treatment, center and baseline included as a covariate. **Results:** Compared with placebo plus SU, the 15 and 30 mg QD doses of PIO + SU lowered HbA_{1c} by 0.88% points and 1.3% points and Tg by 16.6% and 26.1 %, raised HDL-C by 5.1% and 12.2% respectively, and had no significant effect on total-C or LDL-C. The results for AIP are shown below:

	Baseline AIP	Mean Δ AIP	SEM	P-value vs Baseline	P-value vs placebo
Placebo + SU	0.33	+0.01	0.013	P=0.38	
PIO 15 mg + SU	0.35	-0.07	0.013	P=0.0001	P=0.0001
PIO 30 mg + SU	0.35	-0.14	0.013	P=0.0001	P=0.0001

PIO 30 mg QD + SU was superior to PIO 15 mg QD + SU in providing an improved AIP ($P=0.0001$). **Conclusions:** Pioglitazone significantly reduced AIP, in a dose-dependent manner, in patients with T2DM on sulphonylurea. Because of the inverse relationship between AIP and LDL particle size, this decrease in AIP may be extrapolated to reflect an increase in LDL particle size.

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ROSIGLITAZONE IMPROVES GLYCAEMIC AND INSULINAEMIC RESPONSES IN SUBJECTS WITH IMPAIRED GLUCOSE TOLERANCE

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Background and Aims: Rosiglitazone (RSG) reduces the glycaemic response to a mixed meal tolerance test (MTT) in patients with Type 2 diabetes, but has not previously been studied in subjects with IGT. **Materials and Methods:** In a double-blind, randomised study, 18 subjects with persistent IGT received either RSG 4 mg bd or placebo for 12 weeks and underwent an OGTT and MTT to derive post-prandial AUC profiles. **Results:** Data are presented as mean or, for change from baseline, mean ± SD and % change ($^{\dagger}0.05 \leq p < 0.10$; $^*p < 0.05$; $^{**}p < 0.01$):

	Placebo (n=9)	RSG (n=9)
OGTT: glucose AUC (0-3 h) (mmol/l* ^h)	Baseline 27.5 Change vs baseline 0.4 ± 3.2 (1.1%) Change vs placebo -3.6*	27.8 -3.4 ± 3.8 (-12.5%)*
OGTT: insulin AUC (0-3 h) (pmol/l* ^h)	Baseline 1617 Change vs baseline 66 ± 666 (2.9%) Change vs placebo -665 [†]	1698 -608 ± 601 (-33.4%)*
MTT: glucose AUC (0-4 h) (mmol/l* ^h)	Baseline 26.0 Change vs baseline 2.7 ± 4.3 (10.7%) Change vs placebo -3.2*	27.3 -1.6 ± 2.4 (-5.8%)
MTT: insulin AUC (0-4 h) (pmol/l* ^h)	Baseline 981 Change vs baseline 77 ± 179 (9.8%) Change vs placebo -354**	1203 -326 ± 274 (-27.5%)*

Of the 9 subjects in the RSG group, 8 improved their 2-h OGTT glucose values, 4 developed normal glucose tolerance and 5 retained IGT ($p=0.007$ vs placebo). In the placebo group, 1/9 subject worsened to diabetes and 8/9 retained IGT. **Conclusions:** Consistent with its effects in reducing insulin sensitivity, RSG substantially improves the glycaemic and insulinaemic response to OGTT and MTT in subjects with IGT.

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ROSIGLITAZONE MAY REDUCE INSULIN RESISTANCE-RELATED CARDIOVASCULAR DISEASE RISK IN TYPE 2 DIABETES PATIENTS

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Background and Aims: Insulin resistance, as estimated by the Homeostasis Model Assessment (HOMA), has been reported to be an independent predictor of cardiovascular disease in Type 2 diabetes mellitus. One unit increase in log HOMA-IR was associated with a 55% increase in cardiovascular disease during a 4-year follow-up of 960 patients from Verona, Italy. **Materials and Methods:** The effect of rosiglitazone (RSG), a PPAR γ agonist, on insulin resistance was studied in Type 2 diabetic patients participating in three trials. In a 52-week trial, RSG was compared with glyburide (GLYB) as monotherapy. In two 26-week trials, RSG, in combination with maximal doses of sulphonylurea (SU) or metformin (MET), was compared with SU or MET alone. **Results:** The results are summarised in the Table. **Conclusions:** The RSG-mediated decrease in insulin resistance in Type 2 diabetes patients translate into a 9% to 27% reduction in cardiovascular disease. Long-term interventional trials involving RSG are currently underway to assess whether the amelioration of insulin resistance produces a reduction in cardiovascular events.

Regimen (no. of subjects)	Mean change in log HOMA-IR from baseline (95% CI)
RSG 4 mg/day (152)	-0.270 (-0.358, -0.181) $p < 0.0001$
RSG 8 mg/day (146)	-0.491 (-0.578, -0.404) $p < 0.0001$
GLYB (158)	0.030 (-0.043, 0.103) $p = \text{NS}$
RSG 2 mg/day + SU (198)	-0.162 (-0.236, -0.088) $p < 0.0001$
RSG 4 mg/day + SU (183)	-0.344 (-0.425, -0.263) $p < 0.0001$
SU (192)	-0.005 (-0.074, 0.064) $p = \text{NS}$
RSG 4 mg/day + MET (115)	-0.220 (-0.317, -0.122) $p < 0.0001$
RSG 8 mg/day + MET (109)	-0.389 (-0.500, -0.278) $p < 0.0001$
MET (113)	-0.063 (-0.133, 0.008) $p = \text{NS}$

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ROSIGLITAZONE REDUCES C-REACTIVE PROTEIN, A MARKER OF SYSTEMIC INFLAMMATION IN TYPE 2 DIABETIC PATIENTS

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Background and Aims: Markers of systemic inflammation have been associated with risk of progression to diabetes and the development of cardiovascular disease. Included amongst these markers are C-reactive protein (CRP) and white blood cell count (WBC). We set out to determine the effect of rosiglitazone (RSG) on these markers in diabetic patients.

Materials and Methods: From a group of >350 patients who completed a study comparing RSG 4 mg/day and 8 mg/day with placebo (PBO), CRP and IL-6 were measured from frozen samples obtained at baseline and following 26 weeks' treatment. Effects of RSG and PBO on WBC, CRP and IL-6 were assessed.

Results: At baseline, CRP correlated significantly ($p < 0.05$) with gender ($r = 0.275$), BMI ($r = 0.300$), WBC ($r = 0.276$), and log HOMA%S ($r = -0.217$), a measure of insulin resistance. After 26 weeks, treatment with PBO was associated with small, non-significant decreases from baseline in CRP (-13.9% [95% CI, -26.7, 1.2]). Conversely, statistically significant decreases from baseline were seen at both RSG doses without evidence of a dose response (-38.1% [95% CI, -44.8, -30.6] for the combined RSG treatment groups). Differences from PBO were also significant (-30.3% [95% CI, -42.2, -16.0]). The mean % change (Δ) from baseline in IL-6 was small and similar in the RSG and PBO groups (-5.8% RSG, -5.0% PBO). There was a small mean decrease in WBC on RSG ($-0.59 \times 10^3/\text{mm}^3$) but not on PBO. In RSG-treated patients, Δ CRP correlated significantly with Δ IL-6 ($r = 0.5601$) and Δ WBC ($r = 0.2927$). Weaker but statistically significant correlations were observed in the RSG group between Δ CRP and Δ weight, Δ FAs, and Δ fasting insulin; and in the PBO group between Δ CRP and Δ IL-6.

Conclusions: RSG treatment is associated with reductions in the pro-inflammatory markers CRP and WBC. These effects may be related to change in insulin resistance or to inherent anti-inflammatory properties of RSG and could have beneficial consequences for metabolic progression and long-term CVD risk.

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COMBINED EFFECTS OF ROSIGLITAZONE AND ATORVASTATIN ON THE DYSLIPIDAEMIA ASSOCIATED WITH TYPE 2 DIABETES

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Background and Aims: Type 2 diabetes is commonly associated with a complex dyslipidaemic profile. This study evaluated the effects of treatment with rosiglitazone (RSG) and add-on 3-hydroxy-methylglutaryl CoA reductase inhibitor therapy on the lipid profile of patients with Type 2 diabetes. **Materials and Methods:** Lipid-lowering agents were withdrawn for 4 weeks prior to 8 weeks of single-blind treatment with RSG 4 mg twice daily. Patients with LDL-cholesterol (LDL-C) <160 mg/dl and triglycerides <500 mg/dl were then randomly allocated to the addition of placebo or atorvastatin (ATV) 10 mg or 20 mg once daily for 16 weeks. Complete lipid profile and density gradient ultracentrifugation were obtained at 0, 8 and 24 weeks. **Results:** There was an 8% increase in LDL-C during the first 8 weeks of the study. Thereafter LDL-C levels remained stable in patients taking RSG alone. Compared with RSG alone, LDL-C decreased by -33% and -40% in those taking ATV 10 mg and 20 mg respectively. In each group, changes in LDL-C were mirrored by changes in apolipoprotein B after 24 weeks. Improvements in LDL density (R_f) were greatest in the first 8 weeks of the study when patients were taking RSG alone. After 16 weeks of add-on ATV, improvements in LDL density were similar to those seen with RSG alone. With RSG alone, a 7% increase in HDL-C was associated with a 17% increase in HDL2 by week 8. Compared with RSG alone, HDL-C increased by 6-7% in the patients taking add-on ATV. After 16 weeks of add-on ATV, there was a minimal change in HDL2 levels compared with RSG alone. The proportion of patients reporting adverse events was similar for all treatment groups. **Conclusions:** RSG causes a potentially favourable, antiatherogenic change in LDL and HDL heterogeneity in patients with Type 2 diabetes. In addition, ATV-mediated improvements in cholesterol subfractions are sustained when added to RSG treatment.

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OSTEOPENIA IN DIABETES MELLITUS: CORRELATIONS TO IGF-1, IGFBP-1, IGFBP-3, IGFBP-4 AND IGFBP-5.

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Background and Aims: Osteopenia independent of age has been shown in the foot and the hip in diabetic patients. The cause of this is not clear. IGF-1 has important effects on bone metabolism and in diabetes mellitus components of the IGF-1 system are altered. IGF binding proteins modulate IGF actions. IGFBP-1 and IGFBP-3 inhibit IGF's biological activity in general and IGFBP-4 inhibits and IGFBP-5 stimulates IGF-1 actions in bone. The aim of this study was to correlate bone density measured by DEXA Dual energy X-Ray Absorptiometry) to IGF-1 and the binding proteins IGFBP-1, -3, -4 and -5.

Materials and Methods: 26 patients, 8 type 1 and 18 type 2 diabetics, were randomly selected for examination with DEXA and blood sampling. IGF-1 and IGF binding proteins were analysed using radioimmuno assays.

Results: Serum levels of IGF-1 were positively correlated to bone density in femoral neck (T-value) ($r=0.3$, $p<0.01$) and to IGFBP-5 ($r=0.4$, $p<0.02$). There was a negative correlation between bone density in spine (T-value) and IGFBP-1 ($r=-0.4$, $p<0.02$). The females had higher IGFBP-1 levels than the males ($p<0.01$). Type 1 diabetics had higher IGFBP-1 levels ($p<0.008$) and lower IGF-1 levels ($p<0.02$) than subjects with type 2 diabetes. The duration of diabetes was inversely related to IGF-1 levels ($r=-0.2$, $p<0.03$) and positively related to IGFBP-1 levels ($r=0.4$, $p<0.001$).

Conclusions: Osteopenia measured as low bone density was associated with low IGF-1 and IGFBP-5 and high IGFBP-1 levels. This indicates that changes in the IGF system may influence bone metabolism in diabetes. Impaired metabolic control including insulin deficiency may explain the low IGF-1 and high IGFBP-1 level

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THE POSTMENOPAUSE IN DIABETES: BONE QUANTITY AND QUALITY

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Background and Aims: Diabetes mellitus is known to influence bone metabolism in different ways. Photon absorptiometry estimates bone mass whereas ultrasonometry is believed to reflect both bone mass and architecture. We attempted to investigate diabetes' effects according to bone composition and loading in the postmenopausal phase.
Materials and Methods: We studied 74 postmenopausal women (37 with type II diabetes and 37 normal ones with similar anthropometric characteristics by person to person matching). In diabetic women age was 60.8 ± 4.6 years (mean ± 1 SD), years since menopause (YSM) 9.5 ± 4.4 and BMI 27.3 ± 4.4 kg/m² while in normal women 59.9 ± 5.2 , 8.1 ± 4.7 and 26.9 ± 4.1 respectively. Duration of diabetes was 8.3 ± 4.2 years. Bone mineral density (BMD) was measured at L2-L4, proximal femur (PF) by the DEXA method and at Os Calcis (OC) by DEXA and ultrasonometry (BUA, SOS). None of the women had received any medication or suffered from any disease affecting bone metabolism. **Results:** No significant differences were observed between the 2 groups in regard either to absolute or age adjusted BMD values of L2-L4 or PF areas. Calcaneal BMD was significantly higher in diabetic women (0.58 ± 0.1 vs 0.50 ± 0.1 g/cm², $p=0.01$) while neither BUA nor SOS differed significantly. In normal women a significantly positive correlation was observed between PF, OC BMD or BUA and BMI ($r=0.35$ to 0.41 , $p<0.05$) while in diabetic ones only regarding OC BMD and BMI ($r=0.41$, $p<0.05$). Duration of diabetes correlated positively to vertebral and PF BMD ($r=0.31$ to 0.33 , $p<0.05$). No correlation were observed between either HbA_{1c} or osteocalcin and BMD, BUA or SOS in diabetic women. **Conclusions:** Postmenopausal women with type II diabetes do not differ in their bone density of either trabecular or mixed bone compared to normal ones. In contrast loaded trabecular bone mass is augmented in diabetic women but its architecture as reflected by ultrasonometry seems to lack this benefit. In diabetic women obesity affects positively bone mass of loaded trabecular bone. Glucose control does not appear to influence any of the absorptiometric or ultrasonometric parameters.

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ANDROGENS AND SEX HORMONE BINDING GLOBULIN IN WOMEN WITH TYPE 1 DIABETES.

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Background and Aims: Insulin has been demonstrated to stimulate adrenal and ovarian androgen production both *in vitro* and *in vivo*. Insulin also downregulates sex hormone binding globulin (SHBG) which modulates androgen delivery to tissues. Therefore, the aim of this study was to analyse androgenic profile and SHBG in a group of type 1 diabetic women and to assess whether androgens and/or SHBG were influenced by insulin dose and/or glycaemic control. **Materials and methods:** 29 type 1 diabetic women aged 50 (46-60) years [median (percentiles 25-75)] with diabetes duration of 23 (14-33) years and body mass index (BMI) 23 (21-27) kg/m² were studied. C peptide levels were 0.05 (0.03-0.07) pM and HbA_{1c} was 9.0 (7.9-9.7)%. Daily insulin dose was 0.53 (0.46-0.58) U/kg BW. Pre- or postmenopausal status was assessed on the basis of estradiol, LH and FSH. Antiadrenal antibodies were negative. No women received estrogenic treatment. Plasma levels of androstenedione (Δ_4), dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEA-S), total testosterone (TT) and SHBG were evaluated. **Results:** Steroid hormones were not increased in this group: thus Δ_4 , DHEA, DHEA-S and TT were 2.6 (2.0-3.1) nM, 7.0 (3.3-10.6) nM, 3.0 (1.9-3.6) pM and 1.4 (0.9-1.8) nM, respectively. Univariate statistical tests indicated an inverse correlation of Δ_4 ($P=0.005$) and DHEA-S ($P=0.003$) with age. There was no association of androgens with diabetes duration, insulin dose and/or glycaemic control. SHBG was 82 (67-110) nM (normal range: 35-90) and was negatively correlated with BMI ($P=0.05$), daily insulin dose ($P=0.03$) and HbA_{1c} ($P=0.05$). When major confounding factors were considered in a stepwise multivariate analysis, we found a decrease of Δ_4 ($P=0.038$) with postmenopausal status and of DHEA-S ($P=0.012$) with age, while SHBG remained negatively associated with HbA_{1c} ($P=0.047$). **Conclusions:** In type 1 diabetic women, adrenal androgens decrease with age and/or postmenopausal status, without influence of insulin treatment or glycaemic control. However, SHBG levels are inversely correlated to quality of glycaemic control.

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MENSTRUAL DISTURBANCES IN PATIENTS WITH TYPE 1 DIABETES MELLITUS.

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BACKGROUNDS AND AIMS: Menstrual abnormalities are more common in diabetic women. We studied pituitary-ovarian system in patients with type 1 diabetes mellitus (DM).

MATERIALS AND METHODS: We examined 105 patients aged 15-25. The control group included 20 healthy women of similar age. The patients were divided into two groups: 1) patients with regular menstrual cycles (n=62) 2) patients with amenorrhea (n=43). Clinical, gynecologic examination was performed; FSH, LH, prolactin (PRL), estradiol and progesterone levels were measured radioimmunologically. The patients were inspected with due regard of menstrual cycle: women with regular periods - between day 6-9, 13-14 and day 20-23 of menstrual cycle, those with amenorrhea being examined twice, with 7-10-day interval.

RESULTS: The more pronounced disorders, that is low level of FSH (3.8 ± 0.68 IU/l), LH (2.3 ± 0.51 IU/l) and estradiol (117.8 ± 32.6 pmol/l) have been seen in patients with amenorrhea. PRL level was confidently lower than the one in the control group (139.4 ± 19.7 vs 242.4 ± 14.5 mIU/l, $p<0.01$). In patients with regular menstrual cycles in compare with control group the reduced level of FSH (3.8 ± 0.5 vs 7.7 ± 1.0 IU/l, $p<0.01$), LH (2.9 ± 0.42 vs 8.1 ± 2.2 IU/l, $p<0.01$) and estradiol (237.6 ± 57.2 vs 620.2 ± 60.9 pmol/l, $p<0.01$) has been revealed at the phase of ovulation. PRL level was comparable to the control one. Progesterone at the luteal phase was lower versus the one in the control group (5.7 ± 1.49 and 13.8 ± 2.67 nmol/l respectively, $p<0.05$).

CONCLUSIONS: Thus, the findings imply that DM affects both pituitary and ovaries. Low PRL level can also be the evidence for disorders in hypothalamus-pituitary axis. More severe DM was typical for patients with amenorrhea than for those with regular ones. Strict control of DM in some patients with amenorrhea led to restoration of menstrual cycles, but not in all women. Hence, the long-term DM control is the primary task in correction of menstrual function disorders, but in the view of its insufficiency we recommend the estrogen therapy for the rehabilitation of the menstrual cycles.

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ALDOSTERONE AND CORTISOL DYNAMICS DURING ORAL GLUCOSE TOLERANCE TEST IN MILD TYPE II DIABETES MELLITUS

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Background and Aims: The aim of this study was to evaluate the dynamics of plasma aldosterone (AL) and cortisol (CL) during the standard oral glucose tolerance test (OGTT) in patients (pts) with early mild, uncomplicated type II diabetes mellitus (DM). **Material and Methods:** Study group 1 was composed of 30 normal subjects (13-male), mean age = 50.90 ± 2.05 . Study group 2 consisted of 25 pts with type II DM (12-male), mean age = 48.72 ± 1.66 . Mean DM duration was $=5.44 \pm 0.61$ years. Patients with obesity, large vessel atherosclerotic disease, hyperlipoproteinaemias, renal failure or other severe DM complications were excluded from this study. Hormones were determined by radioimmunoassay. **Results:** In normal subjects the AL level at 1st OGTT hour was lower than at OGTT start ($p < 0.05$). At 2nd OGTT hour the AL level came back to initial level. In pts with DM the AL level at 1st OGTT hour did not differ statistically from the one at OGTT start. At 2nd OGTT hour the AL level came back to initial level. In pts with DM the AL levels during OGTT hour did not differ. In normal subjects the CL level at 2nd OGTT hour was lower than at OGTT start ($p < 0.05$). In pts with DM the CL levels during OGTT hour did not differ. In DM the levels of CL at 1st OGTT hour were statistically higher than in normal subjects ($p < 0.05$).

OGTT time	ALDOSTERONE		CORTISOL	
	Normal (pmol/l)	Type II DM (pmol/l)	Normal (nmol/l)	Type II DM (nmol/l)
0 hrs	329.44 \pm 54.74	245.16 \pm 33.40	388.03 \pm 28.31	393.15 \pm 38.15
1 hrs	176.23 \pm 23.94	202.50 \pm 23.40	308.91 \pm 34.32	439.87 \pm 50.33
2 hrs	205.45 \pm 22.76	204.35 \pm 24.02	301.92 \pm 28.07	387.15 \pm 42.05

Conclusions: The results obtained suggest that in type II DM patients the OGTT glucose load does not suppress the secretion of such anti-insulin hormones as aldosterone and cortisol.

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A COMPARISON OF INSULIN LISPRO MIX 25 AND HUMAN INSULIN 30/70 IN THE TREATMENT OF TYPE 2 DIABETES DURING RAMADAN

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Background and Aims: Ramadan is a basic principle of Islam that is strictly observed by approximately 1 billion people worldwide. Ramadan occurs on the ninth month of the Islamic year when followers of Islam must fast from dawn to sunset for one lunar month. In addition to changes in dietary pattern, there may also be an increased intake of fat and carbohydrates. These changes in dietary habits can result in large postprandial glucose (PPG) excursions that have been linked to cardiovascular morbidity in patients with type 2 diabetes. The primary objective of this study was to compare insulin lispro Mix25 (Mix25) and human insulin 30/70 (30/70) in evening postprandial glucose (PPG) control in patients with type 2 diabetes who wished to fast during and during Ramadan.

Materials and Methods: Mix25 and 30/70 were compared in an open-label, randomised, crossover study involving 151 outpatients who had received insulin therapy for at least 2 months prior to entering the study. Mix25 was administered immediately before the morning and evening meals; 30/70 was administered before the same meals using the patients' usual pre-study injection-to-meal interval. Each treatment period had a duration of 14 days. Blood glucose was self-monitored before and 2 hours after the morning and evening meals on 3 days within the last 5 days of each treatment period.

Results: The 2-hour PPG excursion following the main evening final meal after sunset was significantly lower ($p=0.002$) with Mix25 (3.4 ± 2.9 mmol/l) compared with 30/70 (4.0 ± 3.2 mmol/l). The evening pre-meal fasting blood glucose values were also significantly lower ($p=0.042$) with Mix25 (7.1 ± 2.2 mmol/l) compared with 30/70 (7.5 ± 2.6 mmol/l).

Conclusions: During Ramadan, treatment with Mix25 resulted in better glycemic control before and after the evening meal compared with treatment with 30/70. Therefore, patients with type 2 diabetes who fast during Ramadan, may be better managed with Mix25.

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COMPLEXITY OF INFECTIONS IN PATIENTS WITH DIABETES MELLITUS TYPE 2 NOT REFLECTED BY PATTERNS OF ANTIBIOTIC DRUG USE

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Background and Aims: It's general belief that diabetes mellitus patients are more susceptible to more severe and complex infections, although this is not supported by strong evidence. The aim of the study was to analyse and compare patterns of antibiotic drug use in diabetes mellitus type 2 patients in order to identify possible patterns of antibiotic drug use reflecting complexity of infections. **Materials and Methods:** In this cohort study, 2,771 incident diabetes mellitus type 2 patients and 2,771 age, sex, index date and pharmacy matched nondiabetic subjects were identified in the PHARMO Record Linkage System, comprising pharmacy records and hospitalizations for all 320,000 residents of six Dutch cities. Patterns of antibiotic drug use were evaluated with a first-order Markov model. **Results:** From this cohort, 1,765 (63.7%) diabetes mellitus type 2 patients and 1,660 (59.9%) nondiabetic subjects received at least one antibiotic drug between 1991-1999. Initiation of antibiotic drug use was slightly lower in diabetes mellitus type 2 patients (63.1%) compared to nondiabetic subjects (67.1%). Switching of antibiotic drugs was significantly lower in diabetes mellitus type 2 patients (45.1%) compared to nondiabetic subjects (49.0%). More repeats of antibiotic drug prescriptions were observed in diabetes mellitus type 2 patients (55.0%) compared to nondiabetic subjects (51.0%). **Conclusions:** Although we applied various in depth methods to identify possible differences, the results of this study did not show any important differences in antibiotic drug use between diabetes mellitus type 2 patients and nondiabetic subjects. The small differences we found were probably not clinically relevant and therefore do not confirm earlier findings of occurrence of more serious infections and more episodes of infections among diabetes mellitus type 2 patients.

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ULTRASONOGRAPHIC ASSESSEMENT OF SUBCUTANEOUS LIPOHYPERTROPHY AT INSULIN INJECTION SITES.

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Background and Aims: The objective of the study was to evaluate the usefulness of the ultrasonographic (USG) examination for the diagnosis and description of morphological picture of subcutaneous lipohypertrophy resulting from insulin injections. **Materials and Methods:** The study group included 30 patients (16 men and 14 women) with diabetes (10 patients with type 1 diabetes, 18 with type 2 diabetes and 2 with secondary diabetes), aged mean 60 ± 2 (SEM) yrs, with BMI mean 27 ± 0.8 kg/m². All the patients received insulin (10: highly purified porcine insulin, 20: biosynthetic human insulin) for the period mean 9.8 ± 1.7 yrs. Most often they injected insulin twice daily (21) at sites easily accessible to injection but did not follow the recommended rotation system. In all the patients lipohypertrophy was initially defined as visible and palpable increase in subcutaneous fat. All the lipohypertrophied sites were assessed using the ultrasonograph of the Power Vision Toshiba with high frequency linear transducer (7,5 MHz). **Results:** The USG examination visualized lesions at 34 sites, and were qualified as hypertrophy of the adipose tissue, vascular proliferation, and fibrosis: out of those 7 sites showed only lipohypertrophy, 5 sites disclosed lipohypertrophy and angiogenesis, 5 sites presented lipohypertrophy and fibrosis, at 11 sites all the three processes were noted, at 3 sites only fibrosis was present and 3 sites showed vascular proliferation and fibrosis. In summary, the USG examination showed that the most frequent sites of the above mentioned lesions were the abdominal wall ($n=21$), followed by the thighs ($n=7$) and the upper arms ($n=6$).

Conclusions: The USG examination of the subcutaneous tissue is an objective nontraumatic assessment of the presence and type of morphological lesions occurring at sites of insulin-induced lipohypertrophy, suitable for the follow-up its progress.

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INCIDENCE OF COLON CANCER AMONG PATIENTS WITH TYPE II DIABETES MELLITUS

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Background and Aims: The pathophysiology and etiology of colorectal cancers are not well-established but it is thought that hypertriglyceridemia, long colonic transit time, presence of excess bile acids in the intestine, obesity and hyperinsulinism, which may accompany diabetes mellitus, may be involved in development of colorectal cancer. The aim of this study was to determine whether incidence of colorectal cancer was high among patients with type II diabetes mellitus. **Materials and Methods:** Threethousandsixteen patients with type II diabetes mellitus and randomly selected 300 controls were included in the study. Tests for carcino-embryonic antigen and carbohydrate antigen 19-9 were carried out and fecal occult blood determinations performed once daily for three consecutively days. When the results were not in normal ranges, patients underwent colonoscopy and biopsies were obtained when necessary. **Results:** Carbohydrate antigen levels were abnormal in four diabetics and three controls. Levels of carcino-embryonic antigen were found to be abnormal in eight diabetics but it was so in only two controls ($p < 0.05$). Similarly, fecal occult blood test was positive in 43 diabetics but it was so in 19 controls ($p < 0.05$). Fortyfour diabetics and 20 controls underwent colonoscopy. Eight diabetics had colorectal cancer but only one control patient had colorectal cancer. After adjustment for age, body mass index and smoking habit, relative risk was estimated to be 6.98 (95% CI= 0.928-56.284, $p < 0.05$). **Conclusions:** Diabetics were found to have a high incidence of colorectal cancer. Therefore, they should be screened with tests for fecal occult blood test and carcino-embryonic antigen on a regular basis.

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Clinical Variability of Familial Partial Lipodystrophy (Kobberling-Dunnigan Syndrome) in 3 Families from the Ribeirão Preto Region, São Paulo, Brazil
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Background and Aims: Familial partial lipodystrophy, first described by Dunnigan (1974) and Kobberling (1975), is a rare syndrome with only a few families reported since then.

Materials and Methods: Description and clinical characterization of 14 patients that belong to 3 generations of 3 different families with FPL from the Ribeirão Preto region, São Paulo, Brazil.

Results: All patients were females (8 adult/6 children) ranging in age from 3 to 59 years. Two families appear to be of the type 2 variant. The other family most closely resembles the type 1 variant. Besides lipodystrophy, all adult patients present an androgenic phenotype, phlebomegaly, muscular hypertrophy, waist-hip ratio > 0.85 , breast hypotrophy, and hyperinsulinemia. None of the patients is obese. Broad and round faces, excessive fat on the face, neck and supraclavicular fossae, acanthosis nigricans, menstrual irregularity, diabetes mellitus, hyperlipidemia, altered liver function, and hyperandrogenism occur to varying extents among families. Alopecia was observed in all of the adult members of one family (type 1 variant). In children, the more evident clinical findings were hyperinsulinemia and acanthosis nigricans. The latter manifestation was detected in all children of one type 2 FPL family.

Conclusions: These observations suggest that FPL has an extremely variable clinical spectrum. Alopecia found in one of the families represents a new finding of this syndrome, since it has never been described before in FPL patients. Considering that the clinical expression of this syndrome usually starts after puberty, and before this period clinical signs of the disease are less evident, the measurement of insulin levels in children of familial partial lipodystrophy families seems to be useful for the early identification of affected individuals.

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Experimental Glucose-Lowering Agents (In Vitro and Animal Studies)

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EFFECT OF REPEATED ADMINISTRATION OF RWJ241947/MCC-555, A NOVEL INSULIN SENSITIZER, ON SERUM LIPOPROTEIN LEVELS IN db/db MICE
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Background and Aims: Patients with type 2 diabetes commonly have abnormal lipoprotein metabolism characterized by elevated triglycerides (TG) resulting from increased very low-density lipoproteins (VLDL). RWJ241947/MCC-555, a novel insulin sensitizer with modulatory activity at PPAR α and PPAR γ , is under investigation as an oral hypoglycemic agent that improves insulin resistance. The effect of repeated oral administration of this new agent on serum TG, VLDL and VLDL-TG was assessed in db/db mice. **Materials and Methods:** Test drug was given for 2 weeks at daily doses of 1, 3, 10 and 30 mg/kg. Control groups included both db/db and db/+ mice (n=8 in each treatment group). **Results:** Serum TG and lipoprotein levels were significantly higher in db/db than in db/+ mice. Serum TG, VLDL and VLDL-TG levels were consistently and significantly ($p < 0.01$) lower in the db/db 30 mg/kg/d group than in the db/db control group. Serum VLDL and VLDL-TG levels were also significantly ($p < 0.05$) lower in the 10 mg/kg/d group than in the db/db control group. At 30 mg/kg/d, RWJ241947 reduced VLDL-TG to levels similar to those observed in the db/+ control group. Total food consumption throughout the treatment period was greater in the db/db control group than in the db/+ control group, however, no significant difference was found between the db/db control group and the RWJ241947 treated groups.

(mg/dL)	Controls		RWJ241947 (mg/kg/day)			
	db/+	db/db	1	3	10	30
TG	49.9	239.4*	255.8	215.9	153.1	75.3†
VLDL	1350.4	7152.1*	6747.9	4712.7	4110.6†	1943.9†
VLDL-TG	30.4	142.0*	119.2	88.2	59.7†	19.6†

* $p < 0.01$ vs db/+ control; † $p < 0.05$ vs db/db control; ‡ $p < 0.01$ vs db/db control

Conclusion: RWJ241947, a novel insulin sensitizer with modulatory activity at PPAR α and PPAR γ improved mean serum TG, VLDL and VLDL-TG in a dose-dependent manner in db/db mice, suggesting that this agent may be useful in improvement of abnormal VLDL lipoprotein metabolism.

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HYPOGLYCEMIC, HYPERINSULINEMIA-IMPROVING AND HYPOLIPIDEMIC EFFECTS OF A NOVEL INSULIN SENSITIZER IN KK-A^y MICE

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Background and Aims: Insulin resistance is a fundamental pathophysiological factor in the development of type 2 diabetes. RWJ241947/MCC-555, a novel insulin sensitizer that modulates both PPAR α and PPAR γ , is undergoing investigation as an oral hypoglycemic agent. This study compares the metabolic effects of this agent with those of glibenclamide (GLIB), an oral sulfonylurea, and control in KK-A^y mice, an animal model that exhibits hyperglycemia, hyperinsulinemia, hyperlipidemia and insulin resistance. **Materials and Methods:** Male KK-A^y mice (n=5 in each treatment group) were given an oral daily dose of test drug (1, 3, 10 or 30 mg/kg) or GLIB 1 mg/kg for 4 days. Blood was taken on the day after last administration and tested for glucose, insulin, triglyceride (TG), total cholesterol (TC) and free fatty acid (FFA) levels. **Results:** RWJ241947/MCC-555 lowered plasma glucose, insulin, TG and FFA levels in a dose-dependent manner. Significant differences in these variables were consistently observed between the 30 mg/kg/day and control groups. No changes in plasma TC level were observed between the 30 mg/kg/day group and the control group. No significant differences were observed between the GLIB and control groups for any study variables.

Plasma Variable	Test drug (mg/kg/day)				
	Control	1	3	10	30
Glucose (mg/dL)	605	500	425*	400**	290**
Insulin (mU/mL)	345	290	250	180	140*
TG (mg/dL)	720	620	600	480	320*
FFA (mEq/L)	3150	3050	2900	2050**	1450*
TC (mg/dL)	185	195	200	190	160

* $p < 0.05$ vs control; ** $p < 0.01$ vs control

Conclusion: RWJ241947/MCC-555, a novel oral insulin sensitizer which modulates PPAR α and PPAR γ , lowered plasma glucose, insulin, TG and FFA levels in this animal model, whereas GLIB did not.

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GLUCOSE TOLERANCE IN ANIMAL MODELS OF TYPE 2 DIABETES MELLITUS GIVEN TOPIRAMATE

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Background: Preclinical data indicate that the favorable effects of the novel neurotherapeutic agent topiramate (TPM; synthesized from fructose) on biological markers of type 2 diabetes mellitus may occur independently of beneficial effects resulting from a loss of body weight alone. **Materials and Methods:** This study assessed the *in vivo* efficacy of TPM (30 or 100 mg/kg/d) on blood glucose and oral glucose tolerance (OGT) in female db/db mice (7-8 weeks old) treated with TPM orally for 11 days, and on OGT in female Zucker diabetic fatty (ZDF) rats (11 weeks of age) treated with TPM orally for 16 days. **Results:** A significant reduction in fasting plasma glucose (FPG) levels and suppressed elevated blood glucose levels induced by oral glucose challenge was seen with TPM. In db/db mice, FPG (mg/dL±SEM) was 261±24, 188±17, and 160±13 ($P<0.01$ vs. control) for vehicle control and TPM 30 and 100 mg/kg treated groups, respectively ($n=7$ for each group). Blood glucose levels (mg/dL±SEM) at 60 min after oral glucose challenge (2 g/kg) were 448±27, 295±32 ($P<0.05$ vs. control), and 271±53 ($P<0.05$) for vehicle control and TPM 30 and 100 mg/kg treated groups, respectively; whereas at 120 min after challenge, the values were 270±24, 124±20 ($P<0.01$), and 184±32, respectively. In ZDF rats, blood glucose levels (mg/dL±SEM) at 60 min after the oral glucose challenge were 474±25, 342±32 ($P<0.05$ vs. control) and 333±32 ($P<0.01$) for vehicle control, and TPM 30 and 100 mg/kg treated groups, respectively ($n=8$ for each group). At 90 min after oral glucose challenge in ZDF rats, the values were 434±25, 269±32 ($P<0.01$ vs. control), and 242±28 ($P<0.01$), respectively; whereas at 120 min, they were 357±30, 206±26 ($P<0.01$), and 173±23 ($P<0.01$), respectively. **Conclusions:** Oral TPM may improve glucose tolerance and/or insulin sensitivity in these animal models. Further studies will determine the potential efficacy of TPM as an antidiabetic agent in humans.

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NOVEL ANTIOXIDANT L-2264 PREVENTS INSULIN RESISTANCE DEVELOPMENT IN DEXAMETHASONE-TREATED RATS

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Background and Aims: Accumulating evidence suggests a link between increased oxidative stress in diabetes and insulin resistance. The aim of the study was to explore the effect of the new antioxidant L-2264 (heterocyclic amide of phenylpropionic acid) on the insulin resistance development in dexamethasone-treated rats. **Materials and Methods:** Male Wistar rats (3-month-old) were injected dexamethasone (D) (0.125 mg/kg s.c. 13 days). Control rats (C) were given vehicle alone. One group of D-treated animals received L-2264 (200 mg/kg) starting 4 days after the first D-injection and another group was given placebo (CD). At the end of the study fasted rats were subjected to the GTT (3 g/kg i.p.). HOMA algorithm was used to estimate insulin resistance (IR). Oxidative status of experimental animals was assessed by determination of malonic dialdehyde (MDA) contents in liver homogenate. **Results:** At day 14 after the first D-injection there were no differences in basal blood glucose levels between all experimental groups. However, basal hyperinsulinemia was observed in CD rats (211.5±9.2 pmol/l vs C: 67.2±6.2 pmol/l, $p<0.05$). GTT revealed impairment of glucose tolerance in rats after D-administration (AUC/2h over GTT was 1245±52 vs C: 960±48 mmol¹l⁻¹, $p<0.01$). L-2264-treatment prevented D-induced glucose intolerance development reducing AUC over GTT by 40 % ($p<0.05$) in comparison with CD rats. After L-2264 administration basal hyperinsulinemia and IR were significantly decreased (all $p<0.05$) in comparison with CD. In addition, L-2264-supplementation reduced NEFA levels in plasma 2.5-fold ($p<0.05$) and suppressed oxidative stress in diabetic animals reducing MDA contents in liver homogenate 2-fold ($p<0.05$) compared to CD rats. **Conclusions:** This data indicate that L-2264 prevents development of dexamethasone-induced insulin resistance decreasing hyperinsulinemia, NEFA levels and attenuating lipid peroxidation in rats.

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GLYCEMIC CONTROL AND TRIGLYCERIDES IMPROVE WITH TOPIRAMATE IN ANIMAL MODELS OF TYPE 2 DIABETES

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Background and Aims: Topiramate (TPM) is a novel neurotherapeutic agent synthesized from fructose. Reports with Wistar and obese Osborne-Mendel and obese Zucker rats have shown that, in addition to decreasing body weight gain, TPM has a tendency to normalize elevated blood glucose levels, insulin and/or triglycerides. **Materials and Methods:** This study evaluated the *in vivo* efficacy of TPM on blood glucose and triglycerides in nonfasted, diabetic, homozygous female db/db mice (48 days old) treated with either TPM (10, 30, 100, or 300 mg/kg/d; $n=7$ or $n=8$ per group) or rosiglitazone (RSG) (10 mg/kg/d; $n=7$) orally for 11 days (Study 1), and in Zucker diabetic fatty (ZDF) rats treated with TPM (30, 100, and 300 mg/kg/d; $n=7$ or $n=8$ per group) or RSG (3 mg/kg/d; $n=8$) orally for 14 days (Study 2). **Results:** A significant dose-dependent decrease in blood glucose was seen with TPM in Study 1: 27% ($P<0.05$ vs. vehicle control), 35% ($P<0.01$), 37% ($P<0.01$), and 61% ($P<0.01$) for 10, 30, 100, and 300 mg/kg/d TPM, respectively, vs. 66% ($P<0.01$) in the RSG-treated group. TPM also significantly ($P<0.01$) decreased plasma triglyceride levels by up to 42% versus diabetic controls at 100 and 300 mg/kg. In addition, the effect of TPM in db/db mice was not associated with significant body weight changes. In Study 2, TPM significantly decreased blood glucose levels by 25%-50% ($P<0.01$); moreover, the reduction at 30 mg/kg/d occurred without a significant change in body weight. At doses of 30 and 300 mg/kg/d, TPM also decreased plasma triglycerides (20% and 30%; $P<0.05$ and $P<0.01$, respectively). **Conclusions:** These findings in db/db mice and ZDF rats indicate that TPM may be an effective treatment for pathological hyperglycemia, with favorable effects on triglycerides. Further studies will elucidate its potential efficacy in treating type 2 diabetes mellitus in humans.

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A novel insulin-sensitizing agent, JTT-501, inhibits expression of the phosphoenolpyruvate carboxykinase gene via Akt pathway.

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Background and Aims: The expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) is inhibited by insulin and is regulated at the level of gene transcription. In diabetes patients, enhanced hepatic PEPCK expression contribute to an increase in the hepatic glucose output resulting in fasting hyperglycemia. Previous reports showed that some thiazolidinediones inhibit the gene expression of PEPCK by insulin-dependent or -independent mechanism. It has been reported that an isoxazolidinedione JTT-501 (JTT), a novel insulin-sensitizing agent, accelerates insulin signalling cascade from insulin receptor to phosphatidylinositol 3-kinase (PI3-kinase) in adipocytes and skeletal muscle. However, the effect of JTT on the cascade beyond PI3-kinase in the liver has not been investigated. In this study, the effect of JTT on the hepatic PEPCK gene expression and phosphorylation of Akt were examined to evaluate the molecular mechanism of the insulin-sensitizing action of JTT using primarily cultured rat hepatocytes and streptozotocin (STZ)-diabetic rats livers.

Materials and Methods: 1) *in vivo* study; After treatment of STZ-diabetic rats with JTT (100 mg/kg/day) for 7 days, liver samples were dissected. Total RNA and protein were extracted from liver samples. Northern blot analyses of PEPCK and an assay of PEPCK activity were performed. 2) *in vitro* study; Hepatocytes from Wistar rats were isolated by collagenase. Primarily cultured hepatocytes were treated for 8 hr with either 500 microM dibutyl cyclic-AMP (cAMP), 10 nM insulin, 100 microM JTT, 1 microM wortmannin (WRT), or various combinations thereof. Cells were harvested and total RNA and protein were isolated. Northern analyses of PEPCK and western blot analyses of phosphorylated Akt were performed.

Results: Treatment of diabetic rats with JTT decreased mRNA expression and activities of hepatic PEPCK by 60% of those in untreated diabetic rats. JTT decreased the PEPCK mRNA expression in cultured hepatocytes as insulin did. cAMP-induced increase in PEPCK mRNA expression of hepatocytes was also decreased by either JTT or insulin. These effects of JTT on PEPCK mRNA expression were prevented by WRT. The expression of phosphorylated Akt was increased by treatment with JTT, which was also prevented by WRT.

Conclusions: These observations suggest that JTT may exert its anti-diabetic action by decreasing PEPCK gene expression through the PI3-kinase-Akt cascade, which is similar to that by insulin.

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HYPOGLYCEMIC EFFECT OF *Bridelia ndelensis* BARK EXTRACT IN TYPE 2 MODEL DIABETIC RAT

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Background and Aims: *Bridelia ndelensis* is a medicinal plant traditionally used in Cameroon for the treatment of diabetes. To the best of our knowledge there is no published report on the antidiabetic properties of this plant. In this work the hypoglycemic/antihyperglycemic effects of the ethanol extract of *Bridelia ndelensis* bark was explored along with its possible effects on glucose absorption in the gut.

Materials and Methods: Type 2 diabetes was induced by a single intraperitoneal injection of streptozotocin (90 mg/kg body weight) to 48 hour pups of Long-Evans rats. Three groups of rats (n=8 in each group) were used. Extraction was done with 80% ethanol and freeze-dried extract was administered to diabetic rats at a dose of 1.25 g/kg body weight in two prandial states (fasting and simultaneously with glucose at 2.5g/kg). A control group (only water as vehicle) and glibenclamide (5mg/kg) group were included. Blood collected at 0, 60 and 120 min (for fasting) and 0, 30 and 75 min (for postprandial) were analyzed for serum glucose (glucose oxidase method). Inhibition of intestinal glucose absorption was investigated by a timed analysis of the concentration of the sugar after oral load in nondiabetic rats.

Results: The extract had no effect on blood glucose when fed in fasting state (Glucose at 60 min; M ± SEM, mmol/l, Control; 8.90±0.36, Extract: 9.13±0.35 and Glibenclamide: 6.02±0.43; p<0.001 for Control vs Glibenclamide). However, a significant effect was obtained in the glucose fed state at 30 min (Control vs Extract and Glibenclamide: 26.52±1.35 vs 18.03±1.17, and 20.95±1, p<0.05 in both cases). The amount of glucose remaining in the intestine was significantly lower in the extract group compared to Control (M±SEM, mg, 9.82±0.47 vs 1.48±0.48, p<0.001). But, it was not paralleled by an increase in blood glucose level.

Conclusions: The ethanol extract of *Bridelia ndelensis* contain antihyperglycemic principle(s) which does not work through inhibition of glucose absorption from the gut.

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MECHANISM OF HYPOGLYCAEMIC ACTION OF *AVERRHOA BILIMBI* IN STREPTOZOTOCIN-DIABETIC RATS

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Aim: In the present study, we have examined the possible mechanism of the hypoglycaemic action of semi-purified fractions of an ethanolic extract of *Averrhoa bilimbi* Linn (Oxalidaceae) leaves (ABe) in streptozotocin-diabetic male Sprague-Dawley (SD) rats. **Materials and Methods:** Male SD rats aged 10 weeks (200-250g) were made diabetic by injecting a single dose of 60mg of STZ/kg i.p. after a 24-h fast. The leaves of *A. bilimbi* were exhaustively extracted with 80% ethanol, concentrated at 40°C using a rotavapor and partitioned successively with butanol, ethylacetate and hexane to get aqueous (AF), butanol (BuF), ethyl acetate (EF), and hexane fractions (HF). The fractions were freeze-dried to obtain powders of each. The hypoglycaemic property of each fraction (125mg/kg p.o.) was determined by performing OGTT in diabetic rats. To investigate the effect of long term administration of the hypoglycemic fractions, diabetic animals (n=5-6) were treated with vehicle (distilled water), AF (125mg/kg BW) or BuF (125 mg/kg BW), twice a day for 14 days. **Results:** In OGTT, AF and BuF showed marked hypoglycaemic property. The long-term administration of AF and BuF lowered the blood glucose concentration by 38 % (p< 0.05) and 24 % (p<0.05) respectively when compared with vehicle. The serum insulin level was significantly increased in the AF-treated diabetic rats on day 14 when compared to the vehicle-treated diabetic rats (P< 0.05). The hepatic glucose-6-phosphatase activity was significantly lower (P<0.05) in AF- and metformin-treated groups, but not in BuF-treated groups, compared to the vehicle-treated diabetic group. Moreover, there was no change in hepatic glycogen content in AF-, BuF- and metformin-treated groups compared to the vehicle-treated group. **Conclusion:** These results indicate that AF is more potent than BuF in the amelioration of diabetes mellitus in STZ-diabetic rats and therefore a potential source for the isolation of new orally active agent(s) for anti-diabetic therapy.

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Long-term effects of stevioside on the type 2 diabetic Goto-Kakizaki (GK) rats: Potential as a new antidiabetic drug.

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Extracts from leaves of *Stevia rebaudiana* Bertoni (SrB) have been used in traditional medicine in Paraguay and Brazil in the treatment of diabetes. Recently, we demonstrated that one of the constituent, the glycoside stevioside exerts a direct insulinotropic effect in isolated mouse islets and *in vivo* in diabetic rats. **Aims:** To explore if long-term intake of stevioside exerts anti-hyperglycaemic, glucagonostatic or anti-hypertensive effects in diabetic rats. **Methods:** In a controlled, 6 weeks investigation Goto-Kakizaki (GK) rats received 0.025 g/kg BW /day of the glycoside stevioside (purity>99 %) in the drinking water. The tail blood pressure was measured every week. At week 5 an intraarterial catheter was inserted in the rats. After 6 days recovery animals were exposed to an i.v glucose tolerance test (2.0 g/kg BW) and blood samples were drawn during a 180 min period. **Results:** Stevioside caused a suppression of plasma glucose (incremental area under the curve (IAUC)): 985±20 (stevioside) vs 1575±21 (control) mM x 180 min, p<0.05). Corresponding to this stevioside fed animals had enhanced first phase insulin responses compared to controls (IAUC: 343±33 (stevioside) vs 136±24 (control) µU insulin X 30 min, p<0.05). In both groups second phase insulin responses occurred to be steadily increasing during the entire observation period. Interestingly, stevioside caused a suppression of the glucagon level corresponding to the first phase of the insulin response (IAUC: 2026±234 (stevioside) vs 3535±282 (control) pg/ml x 180 min, p<0.05). From week one and onwards a 7 to 9 % decrease in both the systolic (p<0.01) and diastolic (p<0.05) blood pressure appeared in response to stevioside. **Conclusions:** Stevioside normalizes first phase insulin responses and possesses anti-hyperglycaemic, insulinotropic and glucagonostatic effects in the diabetic GK rats. In addition, stevioside caused a pronounced blood pressure suppression. Consequently, stevioside may act as a useful drug in the treatment of type 2 diabetes and the metabolic syndrome.

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INSULIN RELEASING EFFECTS OF SOME PURE COMPOUNDS FROM *PTEROSPERMUM SEMISAGITTATUM* LEAVES ON ISOLATED RAT ISLETS

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Background and Aims: Methanol extract of *Pterospermum semisagittatum* leaves (a plant material used by traditional practitioners for the treatment of diabetes) have earlier been shown to have antihyperglycemic properties both in nondiabetic and diabetic (type 1 and type 2) model rats. Three compounds from the leaves (PS01 & PS02 from methanol extract and PS03 from chloroform extract) have now been explored for their possible effect on insulin secretion *in vitro* from isolated rat islets.

Materials and Methods: The compounds were isolated from *P semisagittatum* leaves by water-acetate or water-butanol partition followed by repeated column chromatography, and those were identified and characterized by spectroscopy. Batches of 8-10 islets (isolated by collagenases digestion from Long-Evans rats) were studied under static incubation at 37°C for 60 min in 400µl HEPES buffered medium (pH 7.4) supplemented with 1mg/ml bovine serum albumin. The compounds, all of which were water soluble, were used at a final concentration of 1mM with 3 or 11mM glucose. Insulin concentration in the medium was measured by an ELISA technique with a rat insulin assay kit.

Results: In response to an increase in glucose conc from 3 mM to 11mM, there was a 3 fold increase in insulin (ng/islet, M±SD; 0.16±0.06 vs 0.45±0.17). The compounds PS01 & PS02 resulted in significant rise in insulin concentration at 3mM glucose (ng/islet, M±SD; control vs PS01; 0.16±0.06 vs 0.36±0.17; p<0.001 and vs PS02; 0.32±0.18, p<0.05). However, at 11mM glucose none of the compounds had any significant effect on insulin release.

Conclusions: The results show that the antihyperglycemic properties of *P semisagittatum* may be, at least, partly due to the insulin releasing properties of the compounds PS01 and PS02.

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EFFECT OF HEMIDESMUS INDICUS ROOT ON PLASMA LIPID AND FREE FATTY ACIDS IN TYPE 2 DIABETIC MODEL RATS

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Background and Aims: Hemidesmus indicus is used in Indian traditional medicine. Recently its hypoglycemic activity was reported, but their effects on plasma lipid in diabetic model rats has not yet been studied. In the present work we have investigated the effect of methanol extract of Hemidesmus indicus (HI) root on plasma glucose, lipid and free fatty acid levels in rats of type 2 diabetic model.

Materials and Methods: Type 2 diabetes was induced by single intraperitoneal injection of streptozotocin (90 mg/kg bw) to 48 hours old pups. The rats (n=9) were fed with freeze-dried methanol extract of HI root (1.25 g/kg bw/10 ml water) once a day for 28 days with consecutive feeding. The Control rats (n=10) were fed only with deionized water. Blood was collected at the beginning of the study period from tail tips and at the end by decapitation of the rats. Plasma glucose, lipid and free fatty acid compositions were measured by enzymatic and gas chromatographic methods respectively.

Results: HI significantly lowered fasting ($p<0.001$) and postprandial plasma glucose ($p<0.001$). It also lowered TG (1.07 ± 0.04 vs 0.73 ± 0.04 ; $p<0.001$), total cholesterol (2.27 ± 0.03 vs 1.82 ± 0.10 ; $p<0.003$), and LDL-C (1.88 ± 0.17 vs 1.49 ± 0.105 ; $p<0.01$) concentrations (mmol/l \pm SD), respectively when compared to Control. However there was no change in HDL-C concentration. The atherogenic ratios (HDL-C:LDL-C, HDL-C:Chol, HDL-C: TG) were increased significantly ($p<0.001$). HI also increased the essential fatty acids (linoleic, acid, linolenic acid, arachidonic acid and docosahexaenoic acid) and decreased the saturated fatty acids (palmitic acid and stearic acid) significantly as compared to the Control.

Conclusions: The results suggest that the methanol extract of HI root affects the lipid and fatty acid level of type 2 diabetic model rats in a manner which counteract atherogenesis.

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Effects of MD-110, a newly synthesized thiocarbamate, on glucose transport in L6 myocytes

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Background and Aims: MD-110, a newly synthesized thiocarbamate, was tested for its glucose transport stimulating-activity using L6 myocytes. As Phosphatidylinositol 3-kinase (PI3K)-dependent signaling, MAP kinase-dependent signaling and calcium-dependent PKC activation were known as the major mediatory routes for glucose transport stimulated by insulin, we investigated the stimulating-mechanism of glucose transport induced by MD-110 using the inhibitors of key enzymes which are involved in the pathways of glucose transport.

Materials and Methods: Glucose transport stimulating effect of MD-110 was evaluated by 2-deoxy-D-[3H]glucose (2-DG) uptake assay and its cytotoxicity was tested by MTT assay. Its stimulating mechanism of glucose transport was investigated by using enzyme inhibitors and calcium modulators.

Results: MD-110 increased the rate of glucose transport in a dose- and time-dependent manner with maximal stimulation at 25 μ M and showed no cytotoxicity at the effective concentrations. The increase in glucose uptake induced by MD-110 was totally inhibited by 10 μ M phenylarsine oxide, a disrupter of trans-Golgi network, indicating that MD-110 enhanced the translocation of glucose transporters from cytosol to plasma membrane. Blockade of protein synthesis by cycloheximide also inhibited glucose uptake in L6 myocytes. Phosphatidylinositol 3-kinase inhibitor and MAP kinase inhibitor didn't exert any significant effect on MD-110-induced glucose transport in L6 myocytes. MD-110-induced increase in glucose uptake into L6 cells was inhibited by intracellular calcium depletion, inhibition of protein kinase C (PKC). Statistical analysis was performed according to Student's t-test.

Conclusions: The above results suggest that MD-110 might enhance glucose transport by the stimulation of glucose transporter synthesis and translocation induced by calcium-dependent PKC activation in L6 myocytes.

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Metabolic Control and Diagnostic Methods

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SUB-OPTIMAL GLUCOSE CONTROL IN HOSPITALIZED PATIENTS. USE OF A SIMPLE ALGORITHM TO BETTER CONTROL

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Background and Aims: Hyperglycemia in hospitalized patients significantly effects the outcome of hospitalization. Even mild elevations of blood glucose have been shown to adversely effect the hospital course and mortality of patients following myocardial infarction, CVA, and cardiac surgery. Data exist which strongly suggest that optimization of glucose control may reduce morbidity and mortality in hospitalized patients. To date few if any proven guidelines for optimization of glucose control on general internal medical wards have been developed. Known diabetics are monitored using finger-stick glucose determinations. Oral medications and insulin are adjusted accordingly. Lack of awareness of the importance of glucose control and fear of hypoglycemia have hindered glucose control during hospitalization.

Materials and Methods: We determined the percentage of hyperglycemia (>250 mg%) and low glucose (<80 mg%) in two general internal medicine wards over a two week period. The staff of both wards were given a one-time instruction in the use of a simple algorithm to improve glucose control using regular insulin: 06:00 to 20:00; glucose of 250-350 mg%: 0.1 unit/kg, and glucose >350 mg%: 0.15 unit/kg. After 20:00 glucose >350 mg%: 0.1 unit/kg. Glucose 250-350 mg% after 20:00, no added RI, NPH only. The percentage of hyperglycemia and low blood glucose before and after use of the algorithm was compared.

Results: Before instruction 71 patients in ward A with 1172 glucose determinations and 28 patients in ward B with 342 determinations had 39% and 29% hyperglycemia respectively, 3% and 1.2% low glucose respectively. After instruction 60 patients in ward A with 576 glucose determinations and 23 patients in ward B with 470 determinations showed a reduction of hyperglycemia to 22% and 21.7% respectively. Percent low glucose rose to 6% and 2.1% respectively.

Conclusions: Use of a simple algorithm can significantly improve hyperglycemia but also increases low blood sugars. Use of analogue insulins might prevent low glucose and further improve control.

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Improving The Treatment Of DKA Using Bedside Capillary Ketone Measurement

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Aims: To define the normalisation time of blood glucose & blood betahydroxybutyrate (BOHB) levels in patients admitted with diabetic ketoacidosis (DKA) and compare this to normalisation of blood glucose and urinary ketones.

Methods: Seven patients (6M and 1F) mean age 36 (range 21 – 46) years admitted acutely with clinical & biochemical features of DKA were studied. All patients were treated using standard guidelines for the management of DKA. Blood BOHB & glucose concentrations were measured using a Precision Optium electrochemical sensor (MediSense/Abbott) and urine ketones with reagent strips (Bayer Pic).

Results: The presenting biochemistry was: glucose 36.6 ± 8.8 mmol/l, (mean \pm SD), pH 7.12 ± 0.11 , venous bicarbonate 9.1 ± 4.5 mmol/l, arterial bicarbonate 6.0 ± 4.4 mmol/l, and BOHB 4.2 ± 0.9 mmol/l. The mean time to normalisation of blood glucose was 9.7 ± 2.1 h and for BOHB 14.9 ± 9.4 h. By comparison urinary ketones only became negative after 53 hours \pm 39, 38 h \pm 30 after normalisation of BOHB concentrations ($p = 0.028$). Assuming patients are converted to a QDS insulin regime, the next injection being after normalisation of both glucose and BOHB at the next usual time (at 0800, 1200, 1600 or 2200 hrs), then iv insulin would be required for 17.7 ± 12 hours, compared to conventional treatment (49.5 ± 10.3 h), potentially saving 34 ± 10.8 h ($p = 0.008$) of iv insulin.

Conclusion: This study shows that in patients with DKA, bedside measurement of blood BOHB, instead of ketonuria, identifies normalisation of metabolism significantly earlier than conventional monitoring. This would allow patients to be safely converted to sc insulin considerably earlier. Wider application of this method would be expected to result in substantial economic savings and patient benefits.

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BLOOD GLUCOSE SELF-MONITORING IN DIABETES CONTROL IN NON-INSULIN TREATED TYPE 2 DIABETIC PATIENTS

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Background and Aims: To investigate the effect of blood glucose self-monitoring (BGSM) on diabetes control in non-insulin treated type 2 diabetic patients. **Materials and Methods:** A total of 250 patients (45–70 years of age, treated with diet or oral antidiabetics) was included in a prospective, multicenter, randomized group-comparison study. Patients allocated to BGSM were asked to measure blood glucose (device with sensor disk) six times (before/after main meals) on 2 days per week (1 working day/1 weekend day) and to record the values measured in a combined blood glucose/eating diary. Patient acceptance was documented over 6 months at 4-weekly visits according to a defined counseling algorithm (4 questions every 8 weeks) and the accuracy of the measurements controlled (wet chemical analysis) or laboratory controls performed (every 8 weeks HbA_{1c}, body weight, lipids, microalbumin). General well-being (WBQ) and treatment satisfaction (DTSQ) were determined before the start and after completion of the study. The intention-to-treat (ITT) analysis comprised 239 patients (122 BGSM) and the per-protocol (PP) analysis 223 patients (113 BGSM). **Results:** HbA_{1c} improved without and with BGSM by a mean of 0.5 ± 1.4 and $1.0 \pm 1.1\%$ points respectively ($p = 0.003$; end values: 7.9 and 7.4; ITT = PP). The quality of life improved markedly with BGSM ($p = 0.053$) and significantly with respect to the sub-items depression and negative well-being ($p = 0.03$). Treatment satisfaction was equal in both groups ($p = 0.9$). In the BGSM group, HbA_{1c} did not improve in 24%, improved continuously from 8.4 to 6.8 in 58% and with delayed success from 8.5 to 8.0 in 18% of the patients. **Conclusions:** Meal-related BGSM within a structured counseling program improves diabetes control in non-insulin treated type 2 diabetic patients.

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WITHIN-SUBJECT VARIATIONS OF PLASMA GLUCOSE AND INSULIN FOLLOWING A SPONTANEOUS MIXED MEAL: CONSEQUENCES FOR THE POSTPRANDIAL GLUCOSE TOLERANCE TEST
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Introduction - It has been suggested that a test meal would be more appropriate than an oral glucose test (OGT) for the diagnosis of diabetes. However, the pattern and the reproducibility of the glucose response to a spontaneous meal has not been established even in non diabetics. In this study, we assessed the relevance of different time points to accurately predict the postprandial glucose and insulin concentrations.

Methods - Subjects were eleven healthy male subjects (mean age 22 ± 1 yrs and BMI 21.7 ± 1.8 kg/m²). On two test days (D1,D2) separated by a week interval, plasma glucose, insulin and free fatty acids (FFA) were measured from lunch up to the following 240 min. Lunch consisted of a pasta dish and a dessert. It was consumed *ad libitum* on D1 and served in the same amount on D2. Using a double lumen catheter, blood was continuously withdrawn and collected with a frequency of 1 tube every 5 min. Tested points were the usual OGT time points (0, 60, 120 mn), the glucose peak level (GPL) and the the lowest coefficient of variation for glucose (GLCV).

Results - Mean energy intake at lunch (EIL) was 3425 ± 196 kJ. The most relevant time point for GPL was 45 mn (46 ± 3 mmol/l, 6.5 ± 0.2 mmol/l). The GLCV was found at 205 mn ($5 \pm 1\%$). The CV of GPL was negatively correlated with EIL, and more specifically the dessert item ($r = -0.81$, $P < 0.005$). This correlation was not found for the other time points. The CV of the area under the curve for glucose (G-AUC) was $8 \pm 1\%$. The 120 mn time point showed the strongest correlation with G-AUC ($r = 0.91$, $P < 10^{-8}$) and allowed the best predictive equation with glucose, insulin and FFA as factors ($r^2 = 0.89$, $P < 10^{-8}$). Using only glucose, the AUC constructed with the 0-45-90 and 120 mn time points provided the most accurate estimation of G-AUC ($r = 0.94$, $P < 10^{-8}$). For insulin, the peak delay was found at 41 ± 2 mn (91.8 ± 12 µU/mL) and the lowest CVs at 45, 80 and 180 mn ($17 \pm 3\%$). Interestingly, insulin and FFA at 45 mn were strong predictive factors of the AUC of insulin ($r^2 = 0.73$, $P < 10^{-5}$).

Conclusion - Measuring glucose 45 min after the onset of a test meal allows to determine the postprandial peak level and improves the evaluation of the glucose response. Its reproducibility is better if the energy intake at the test meal is high. Last, plasma FFA seem an important factor in the glucose and insulin responses to a meal.

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A SINGLE MEASUREMENT OF HbA_{1c} OR FASTING BLOOD GLUCOSE IN THE EARLY PHASE OF TYPE 2 DIABETES PREDICT LONG TERM GLYCAEMIC CONTROL

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Background and Aims: 1) To investigate whether one measurement of HbA_{1c} or fasting blood glucose (FBG) could predict average HbA_{1c} over a 10-year period in type 2 diabetes (T2DM). 2) To study whether HbA_{1c} reflected primarily fasting or non-fasting blood glucose. It has been suggested that achieving glycaemic goals is easier in some patients with T2DM than others irrespective of the treatment given and the efforts used. A study in type 1 diabetes reported that a single measurement of HbA_{1c} predicted long-term glycaemic control. Furthermore, the value of self monitoring of blood glucose (SMBG) in T2DM has been debated. **Materials and Methods:** In a 10-year prospective study we investigated 31 men and 24 women with T2DM. At start, their mean (S.D.) age was 59.3 (6.2) years, duration of diabetes 7.3 (3.1) years, BMI 26.7 (3.7) kg/m², baseline HbA_{1c} 8.6 (1.5) %, and FBG 11.1 (2.6) mmol/l. The patients were originally randomly assigned to start insulin treatment or continue on oral hypoglycaemic agents, but all except two were eventually treated with insulin due to symptomatic hyperglycaemia or HbA_{1c} >10%. We recorded HbA_{1c} and three 4-point SMBG-profiles each six months throughout the study period. **Results:** A single measurement of FBG and HbA_{1c} before treatment allocation, correlated positively and significantly to the mean HbA_{1c} over the 10 year period ($r=0.43$, $p=0.004$ and $r=0.38$, $p=0.01$ respectively). Body mass index, fasting C-peptide levels or insulin sensitivity and b-cell function measured with the homeostasis model assessment at study start, did not show significant correlations to mean HbA_{1c}. Fasting values for SMBG correlated stronger to mean HbA_{1c} over the 10 year period than non-fasting SMBG ($r=0.51$, $p=0.001$ versus $r=0.35$, $p=0.02$), indicating that FBG is a more important determinant of HbA_{1c} than non-fasting blood glucose levels. **Conclusions:** A single measurement of HbA_{1c} or fasting blood glucose in an early phase of type 2 diabetes may indicate which long-term glycaemic control that can be achieved. Levels of HbA_{1c} reflect non-fasting blood glucose levels poorer than fasting levels, emphasising the importance of SMBG given the possibility that postprandial glycaemia may be of importance in the development of diabetic late complications.

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CAN GLUCAGON-STIMULATED C-PEPTIDE LEVEL BE A SUFFICIENT INDICATOR OF SECONDARY FAILURE TO ORAL ANTIDIABETIC AGENTS?
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Aim: The purpose of our study was to evaluate the factors affecting secondary failure to oral antidiabetic agents by assessing fasting and glucagon stimulated 6th minute C-peptide levels. **Material and methods:** One hundred and thirty two type 2 diabetic patients were included in this study and divided into two groups. Group I consists of 104 patients, 35 to 76 years of age, duration of diabetes ranging from 1 to 29 years with secondary failure to oral antidiabetic agents (HbA_{1c}>6.5) and group II consists of 28 patients, 38-73 years of age, duration of diabetes ranging from 1 to 25 years without secondary failure to OAD (HbA_{1c} ≤ 6.5). Measuring C-peptide levels after 12 hours of fasting and the 6th minute of IV bolus injection of 1 mg glucagon assessed beta cell reserve. Correlations between fasting (F-C) and glucagon stimulated C-peptide (S-C) levels and age, the age at onset of diabetes, duration of diabetes, genetic density, BMI, HbA_{1c}, blood pressure, serum lipids were evaluated. **Results:** Increase of C-peptide levels more than 50% of basal levels after glucagon stimulation which is accepted as sufficient for beta cell reserves, were seen in 51% of patients in group I and 75% of patients in group II. When the patients who displayed more than 50% increase in C-peptide levels were compared, the percentage of increase in group 2 was significantly higher than that of in group I (78.86 ± 50.38 vs 56.39 ± 30.93 respectively, $p=0.004$). We found that the duration of diabetes in group I was significantly longer than in group II in patients who had an increase in C-peptide levels more than 50% of basal levels (9.83 ± 6.78 vs 3.24 ± 1.92 years, $p=0.0001$). BMI was positively correlated with F-C and S-C levels ($r=0.321$, $p=0.001$, $r=0.339$, $p=0.0001$ respectively). Patients with BMI<27, had lower F-C and S-C levels (2.56 ± 1.08 , 4.12 ± 1.79 ng/ml, $r=0.43$, $p=0.001$, $r=0.317$, $p=0.018$, respectively). In hypertensive patients, F-C and S-C levels were found to be significantly higher ($r=0.379$ $r=0.404$, $p=0.0001$, respectively). Beta cell reserves were not affected by genetic density, duration of diabetes and age of diabetes onset. **Conclusion:** Secondary failure to OAD can also occur in spite of good beta cell reserve probably due to insulin resistance. Beta cell reserve was not correlated either to the duration of diabetes or the age at diagnosis of the patients. Glucagon stimulated C-peptide levels is not a valuable marker to demonstrate secondary failure to OAD agents even though the response to glucagon stimulation is sufficient.

USEFULNESS OF GLUCOSE TOLERANCE TEST FOR DIAGNOSING DIABETES -ANALYSIS OF 12,156 CASES

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Background and Aims: Recently, the necessity of oral glucose tolerance test (OGTT) to diagnose diabetes has been under discussion. The aim of this study was to estimate the usefulness and indication of OGTT.

Materials and Methods: In 12,156 subjects of our institutes from 1993 to 1999, we analysed body mass index (BMI), fasting plasma glucose (FPG), triglyceride (TG), total cholesterol (TC), hemoglobin A1c (HbA1c) and 75g OGTT. We diagnosed diabetes by the criteria of WHO 1998. Statistical analyses were made by a regression analysis and Student's unpaired t-test.

Results: 744 subjects were diagnosed as diabetes. Surprisingly, 65 % of those patients (477 subjects) showed high 2-h plasma glucose in the OGTT (2h-PGs) (>200 mg/dl) although their FPGs were less than 126 mg/dl. A regression analysis on this group revealed that FPG 103 mg/dl corresponded to 2h-PG 200 mg/dl, indicating that the subjects whose FPGs ranged between 103 to 125 mg/dl (border FPG group) were highly recommended to undergo the OGTT. Interestingly, in the border FPG group, BMI (diabetic, 24.76 \pm 0.15, n = 304; non-diabetic, 23.74 \pm 0.05, n = 2724, P<0.05; mean \pm SEM), TG (diabetic, 184.3 \pm 6.0 mg/dl, n = 304; non-diabetic, 149.79 \pm 1.8 mg/dl, n = 2724, P<0.05; mean \pm SEM), and HbA1c (diabetic, 5.72 \pm 0.04 %, n = 190; non-diabetic, 5.21 \pm 0.01 %, n = 1964, P<0.05; mean \pm SEM), but not TC, of diabetic group (2h-PG \geq 200mg/dl) were significantly higher than those of non-diabetic group (2h-PG < 200 mg/dl) respectively. Another regression analysis showed that BMI 24.2, TG 160 mg/dl and HbA1c 5.5 % corresponded to 2h-PG 200 mg/dl, suggesting that BMI 24.2, TG 160 mg/dl and HbA1c 5.5 % are thought to be useful values for the indication of OGTT.

Conclusions: The diagnosis of diabetes by only the value of FPG would be insufficient and an OGTT is effective for precise diagnosis of diabetes. We recommend the indication of an OGTT for the subjects whose FPG ranged between 103 and 125 mg/dl and whose BMI, TG or HbA1c showed abnormally high value(s).

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Lifestyle and Dietary Interventions

Low-calorie-diet reduces the antilipolytic action mediated by α 2-adrenergic receptors in adipose tissue in obese women: microdialysis study in situ.

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Background and Aims: Alpha2-adrenoceptor (α 2-AR) stimulation was shown to blunt lipid mobilization during exercise in subcutaneous abdominal adipose tissue (SCAAT) in obese men. The aim of this study was to investigate the α 2-AR involvement in the control of exercise-induced lipolysis in SCAAT in obese women and to evaluate whether the α 2-AR-mediated action can be modulated by a low-calorie-diet (LCD).

Materials and Methods: Ten obese women (age 37.2 \pm 5.5, body weight 99.1 \pm 4.6 kg, body mass index 34.3 \pm 1.1 kg/m²) were submitted to LCD (5.5 MJ/day) for 10 weeks. Using the microdialysis method, the exercise-induced α 2-adrenergic antilipolytic effect was investigated in SCAAT before LCD and during the last day of the diet. Changes in extracellular glycerol concentrations (EGC) and blood flow were measured in SCAAT at rest and during 45 min exercise bouts (50% of heart rate reserve) in a control microdialysis probe and in a probe supplemented with the α 2-AR antagonist phentolamine.

Results: Plasma norepinephrine, epinephrine and insulin levels at rest and their exercise-induced responses were not different before and during LCD. Before LCD, the exercise-induced increase in EGC was higher in the probe supplemented with phentolamine compared with the control probe (area-under-curve (AUC): 9211 \pm 1223 vs 5831 \pm 729 μ mol/l/45 min, respectively, p<0.004). During LCD, no differences in the exercise-induced responses between the phentolamine-supplemented and the control probe were found.

Conclusions: These findings suggest that the antilipolytic action mediated by α 2-ARs is involved in the control of exercise-induced lipolysis in SCAAT in obese women. This antilipolytic action is reduced during low-calorie-diet.

The study was supported by a grant of the Trant Agency of the Czech Republic 303/00/0649.

METABOLIC TREATMENT TARGETS ACHIEVED IN PATIENTS WITH DIABETES OVER THE AGE OF 70 YEARS

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Background and Aims: There is limited data on metabolic targets achieved in patients with diabetes mellitus (DM) over 70 years compared to younger populations as in DCCT and UKPDS.

Material and Methods: 567 of 1627 patients attending a secondary care diabetic clinic for annual review are over 70 years and form 5 treatment groups (a)-(e). These 5 groups comprise, according to treatment, and by age (mean \pm SD) yrs and duration of diabetes (mean \pm SD) yrs: (a) 61 patients with type 1 DM, aged 76 \pm 5, duration of DM 24 \pm 15, (b) 39 pts with type 2 DM, diet treated, aged 75 \pm 4, duration of DM 8 \pm 5, (c) 301 pts with type 2 DM on oral agents, aged 76 \pm 5, duration 11 \pm 8, (d) 156 pts with DM requiring insulin, aged 76 \pm 5, duration 17 \pm 9, (e) 10 pts with type 2 DM on insulin and metformin, aged 75 \pm 5, duration of DM 16 \pm 8.

Results: Total cholesterol did not differ between treatment groups, being lowest in group (b) 4.8 \pm 1.0 mmol/l and highest in (e) 5.2 \pm 0.9 mmol/l. Likewise for serum creatinine, lowest in (b) 104 \pm 21, and highest in (d) at 116 \pm 37 μ mol/l. Mean HbA1c increased from 7.3 \pm 1.3 % in group (b) to 8.8 \pm 1.3 % in (e). Mean HbA1c in type 1 patients over 70 years of age (8.8 \pm 1.5%) was the same as in 438 type 1 patients under 70 years (8.8 \pm 1.8%). BMI (kg/m²) increased from 26 \pm 5 in Type 1 patients (group (a)) to 34 \pm 8 in group (e). Conversely the lowest mean systolic BP (142 \pm 19) mmHg and diastolic BP (77 \pm 13 mmHg) were in group (e), and highest (150 \pm 18 and 83 \pm 15 mmHg respectively) in group (b). Type 1 patients over 70 years had mean BP 146 \pm 21/76 \pm 10 mmHg, the systolic pressure being significantly higher (P<0.05) than in Type 1 patients under 70 years (mean 128 \pm 17/78 \pm 9 mmHg).

Conclusion: Metabolic targets achieved in patients with DM over 70 years vary according to treatment groups. They are close to those in younger patients except for systolic BP in Type 1 DM. They are also close to suggested targets, except for %HbA1c which exceeds 7.0% in all groups.

INCREASING FIBRE AND DECREASING FAT INTAKE LOWERS THE BLOOD GLUCOSE CONCENTRATION IN OVERWEIGHT SUBJECTS WITH STABLE WEIGHT

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Background and Aims: Weight reduction is recommended to subjects who are overweight and have high blood glucose. Weight reduction, on the other hand, is difficult to achieve and maintain. The aim of this study was to assess the relationship of dietary changes and glucose tolerance in subjects who were not able to reduce their weight during a two-year follow-up. **Materials and Methods:** 522 overweight subjects with IGT were randomised into the intervention (IG) or the control group (CG) of the Finnish Diabetes Prevention Study (DPS). The main components of the life-style intervention were weight reduction, exercise, and low-fat, high-fibre diet. All subjects received advice about healthy diet and exercise habits; in the IG the intervention was intensive and individualised counselling. An oral glucose tolerance test was carried out and weight measured annually. Subjects with weight change from baseline to both 1-year and 2-year examinations < \pm 3% were classified as 'no-weight-change group' (n=148). In order to control for the effect of exercise on glucose values, subjects were classified into three categories according to leisure time exercise habits: 'no exercise', 'moderate exercise >4 hours/week' or 'conditioning exercise >3 hours/week'. Diet was assessed with 3-day food records, and the changes in intakes from baseline to year 2 were calculated. Differences in glucose concentrations by dichotomised changes in nutrient intakes (median value as cutpoint) were analysed by ANOVA. **Results:** In the lowest exercise category (n=32), the change in carbohydrate intake was negatively associated with the change in fasting (p=0.0154) and post-load (0.0411) glucose, and the change in fat intake was positively associated with the change in post-load glucose concentration (p=0.0386). In the middle exercise category (n=89) the change in total fibre density (p=0.0063), water-soluble fibre density (p=0.0337), and carbohydrate intake (p=0.0276) were negatively, and the change in total fat (p=0.0389) and saturated fat (p=0.0284) were positively associated with the change in post-load glucose concentration. **Conclusions:** If weight reduction cannot be attained while aiming to control high blood glucose values, a significant reduction in blood glucose concentration can still be achieved by decreasing fat intake and increasing carbohydrates and fibre intake.

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INTENSIFIED LIFESTYLE INTERVENTION OR INSULIN TREATMENT IN TYPE 2 DIABETIC PATIENTS WITH FAILURE ON ORAL HYPOLYCAEMIC AGENTS - WHICH IS BEST?

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Background and Aims: The aims of this study was to assess whether, in type 2 diabetic patients with inadequate glycaemic control with oral hypoglycaemic agents (OHA), an intensified lifestyle intervention program 1) was as effective as insulin treatment in controlling blood glucose, blood lipid and blood pressure during one year, and 2) could prevent the weight gain usually accompanying the introduction of insulin treatment in such patients.

Materials and Methods: Thirty-eight subjects with type 2 diabetes treated with OHA, HbA1c 8-10.5% and BMI 26-40 kg/m², were randomized to the following treatments 1) intensified lifestyle intervention (Life), 2) Life + insulin treatment (Life+Ins) and 3) Insulin treatment (Ins). The one-year Life-program consisted of a "lifestyle course" and group exercise twice a week. Data are given as median (inter quartile range).

Results: Twenty-six % of the randomized subjects dropped out because of illness or non-compliance. Among those who completed the intervention according to the protocol, the Life group reduced their weight by 3.0 (4.0) kg ($p < 0.05$), while the Life+Ins and Ins groups increased weight by 3.5 (3.4) ($p < 0.05$) and 4.9 (6.9) kg (n.s.), respectively. Body mass index, total body fat and abdominal fat (measured by DEXA) changed similarly to body weight. There was a significant difference between the changes in the Life group and the two insulin-treated groups in all anthropometric measurements except for changes in fat-free body mass. The Life+Ins showed an increase in fat-free body mass with 1.7 (2.0) kg ($p < 0.05$), while the Life group and Ins group showed no change. The levels of HbA1c were significantly reduced in the Life and Life+Ins groups, by 1.2 (1.0) %-points and 1.0 (1.7) %-points, respectively (both $p < 0.05$). There was a similar, but non-significant trend in the Ins group by 1.5 (2.5). The cholesterol/HDL-cholesterol ratio was lowered in all groups ($p < 0.01$ in groups 1 and 2). There were no significant differences between the three treatment groups in the changes achieved in blood glucose regulation, blood lipids or 24-hour blood pressure during the intervention year.

Conclusions: Intensified lifestyle intervention was as effective as insulin treatment in improving glycaemic control and also in controlling lipids and blood pressure levels in poorly controlled subjects with type 2 diabetes, and prevented the weight gain observed with insulin therapy. The lifestyle intervention program was not able to prevent the weight gain that accompanied insulin therapy.

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ORLISTAT PROMOTES WEIGHT LOSS AND IMPROVES GLYCAEMIC CONTROL IN OVERWEIGHT PATIENTS WITH TYPE 2 DIABETES

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Background and Aims: The effects of treatment with orlistat on body weight, glycaemic control and other cardiovascular risk factors were investigated in a 1-year study of overweight or obese patients with type 2 diabetes. **Materials and Methods:** Overweight or obese adults (BMI > 28 kg/m²) with type 2 diabetes were eligible for inclusion if they had an HbA1c of 6.5-11% and were either newly diagnosed and not receiving any antidiabetic medication or had received sulphonylurea therapy for at least 2 months prior to the study. Patients treated with anti-diabetic medications other than sulphonylureas were excluded. After a 4-week, single-blind, placebo lead-in period, patients were randomised to double-blind treatment with orlistat 120 mg or placebo three times daily in conjunction with a mildly reduced-calorie diet (600 kcal/day energy deficit, $< 30\%$ of calories as fat) for 48 weeks. **Results:** A total of 383 patients were randomised to treatment, of whom 180 in the placebo group and 189 in the orlistat group were eligible for ITT analysis; 65% of patients were sulphonylurea-treated. After 1 year, patients in the orlistat plus diet group lost significantly more weight than patients in the placebo plus diet group (-5.4% vs -3.6%; $p < 0.01$). Moreover, a significantly higher proportion of patients achieved weight loss of 5% or more with orlistat compared with placebo (51.3% vs 31.6%; $p = 0.0001$). Furthermore, reduction in waist circumference was almost 2-fold greater in orlistat-treated patients compared with placebo-treated patients (-5.5 cm vs -3.0 cm; $p = 0.002$). Patients treated with orlistat had significantly greater improvements than placebo-treated patients in HbA1c (-0.9% vs -0.4%; $p < 0.001$), fasting glucose (-1.63 vs -0.71 mmol/L; $p < 0.01$) and postprandial glucose (-1.81 vs -0.53 mmol/L; $p < 0.01$). In addition, patients treated with orlistat achieved a greater reduction in mean daily sulphonylurea dose than patients treated with placebo. Orlistat was also associated with significantly greater improvements than placebo in total cholesterol (-2.3% vs +1.8%; $p < 0.01$) and LDL-cholesterol (-2.0% vs +5.1%; $p < 0.05$). Orlistat had a similar safety profile to placebo, with the exception of a higher incidence of generally mild and transient gastrointestinal events known to be associated with the mode of action of orlistat. **Conclusions:** Weight loss with orlistat in conjunction with a mildly reduced-calorie diet improves glycaemic control and reduces sulphonylurea requirements in overweight or obese patients with type 2 diabetes.

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Intensive Lifestyle Intervention is Necessary to Improve Insulin Sensitivity

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Background and aims: Degree of adiposity, level of physical activity and diet composition are determinants of insulin sensitivity. This study compares the effect on insulin sensitivity of current dietary and exercise recommendations and a more intensive intervention in normoglycaemic insulin resistant individuals.

Materials and Methods: 79 normoglycaemic insulin resistant (determined by the euglycaemic insulin clamp) men and women were randomised to a control group or one of two combined dietary and exercise programmes. One group (modest level) based on current clinical recommendations and the other on a more intensive dietary and exercise programme. Pre and post measurements (at 4 months) included: insulin sensitivity using a euglycaemic insulin clamp, body composition using dual-energy absorptiometry (DXA), aerobic fitness using a submaximal VO₂ test, a 4 day diet record, anthropomorphic measures, fasting glucose, insulin and a lipid profile.

Results: Only the intensive group showed a significant improvement in insulin sensitivity (23% increase compared to 9% in the modest group, $p = 0.01$). This was associated with a greater weight loss (4.6 kg versus 4 kg from total fat and 3 kg versus 2 kg of truncal fat) and a significant improvement in aerobic fitness (11% increase in the intensive compared to 1% in the modest, $p = 0.04$) and a greater fibre intake in the intensive group, but no difference in reported total or saturated dietary fat.

Conclusions: Current dietary and exercise recommendations do not significantly improve insulin sensitivity, however a more intensive programme does. Weight loss and improved aerobic fitness, rather than diet composition appear to be the most important determinants of the programme.

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EFFECT OF ORLISTAT IN OVERWEIGHT AND OBESE TYPE 2 DIABETES PATIENTS TREATED WITH METFORMIN

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Background and Aims: Weight loss has known beneficial effects on glycaemic control and cardiovascular (CV) risk factors in type 2 diabetes (D), but sustained weight reduction is difficult to achieve. Metformin results in less weight gain than insulin or sulphonylureas, and is therefore often used preferentially for obese D patients. The objective of the present 1-year multicenter, randomized, double-blind, placebo-controlled trial was to assess the effect of orlistat (ORL), a gastrointestinal lipase inhibitor, on body weight, glycaemic control and CV risk factors in obese D treated with metformin (1000-2500 mg/d) alone or combined with other oral agents.

Materials and Methods: Patients with a BMI 28-43 kg/m² and an HbA1c of 7.5-12.0% were randomised to a hypocaloric diet plus ORL (n=249) or placebo (PL; n=254).

Results: After 1 year, mean (\pm SEM) weight loss was significantly greater in ORL than PL (-4.6 \pm 0.3% vs -1.7 \pm 0.3% of initial weight; $p < 0.001$), and a greater proportion of ORL achieved 5% weight loss (39.0% vs 15.7%; $p < 0.001$), and 10% weight loss (14.1% vs 3.9%; $p < 0.001$). More ORL compared with PL had a decrease in HbA1c of 0.5% (61% vs 43%; $p < 0.01$) and 1.0% (46% vs 29%; $p < 0.01$). In addition, more patients treated with ORL than PL decreased or discontinued at least one anti-diabetic medication compared with PL (17% vs 8%), while fewer ORL than PL patients increased their medication dose or added another anti-diabetic medication (12% vs 22%); $p < 0.001$ for the difference between ORL and PL in medication changes. Compared with PL, patients treated with ORL had greater decreases in fasting plasma glucose (-2.0 \pm 0.2 vs -0.7 \pm 0.2 mmol/L; $p = 0.001$), total cholesterol (-4.1 \pm 0.9 vs +2.6 \pm 1.0%; $p < 0.0001$), LDL-cholesterol (-2.8 \pm 2.3 vs 3.9 \pm 2.7%; $p < 0.05$), LDL/HDL ratio (-0.60 \pm 0.07 vs -0.46 \pm 0.08; $p < 0.05$), and systolic blood pressure (-2.1 \pm 0.8 vs -0.4 \pm 0.9 mm Hg; $p < 0.05$). Overall, more PL than ORL withdrew prematurely from the study (44% vs 35%, $p < 0.05$); of these patients, more ORL than PL patients (10% vs 5%) discontinued treatment because of an adverse event.

Conclusions: These data demonstrate that orlistat is a useful weight management tool that results in improvement in glycaemic control, lipid profile and blood pressure in obese patients who are being treated with metformin for type 2 diabetes mellitus.

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Latin-American Multicentric Study with Orlistat in Overweight or Obese Patients with Type 2 Diabetes

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Background and Aims: A 6-month, multicentre, randomised, placebo-controlled study was conducted in 5 Latin-American countries in order to assess the effect of orlistat on weight loss, glycaemic profile and other obesity-related cardiovascular risk factors in overweight or obese patients with type 2 diabetes.

Materials and Methods: Patients aged 18-70 years with a BMI >27 kg/m², diagnosed type 2 diabetes, and HbA1c of 6-11% were enrolled into a 2-week single-blind placebo lead-in period, at the start of which they were prescribed a mildly reduced-calorie diet (600 kcal/day energy deficit, 30% of calories as fat). Patients who completed the lead-in were then randomised to treatment with orlistat 120 mg or placebo three times daily in conjunction with diet for 6 months. Concomitant treatment with any antidiabetic medication except insulin or acarbose was permitted during the study.

Results: A total of 365 patients were enrolled, of whom 338 entered double-blind treatment and were eligible for intent-to-treat analysis (orlistat, n=164; placebo, n=174). After 24 weeks, the orlistat plus diet group had significantly greater weight loss than the placebo plus diet group (4.7% vs 3.0%; p=0.0003). Almost twice as many patients receiving orlistat plus diet lost 5% or more of initial body weight compared with placebo plus diet (30% vs 17%; p=0.003). Orlistat was associated with a significant improvement in glycaemic control compared with placebo, as reflected by greater reductions in HbA1c (-0.6% vs -0.2%; p=0.06), fasting plasma glucose (-1.00 vs -0.01 mmol/L; p=0.04) and postprandial glucose (-1.06 vs +0.17 mmol/L; p=0.05). Orlistat was also associated with significantly greater improvements than placebo in total cholesterol (-4.2% vs +1.9%; p=0.0001) and LDL-cholesterol (-3.6% vs +4.7%; p=0.0002). Orlistat was well-tolerated. Gastrointestinal events were more frequent in the orlistat than placebo group (65% vs 37% of patients) but these were generally mild to moderate in intensity, transient and limited to 1-2 episodes per patient.

Conclusions: Treatment with orlistat plus diet promoted clinically significant weight loss and improved glycaemic and lipid profiles in overweight or obese patients with type 2 diabetes.

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GLYCEMIC INDEX, INSULIN INDEX AND NEFA INDEX OF RICE AND CHAPATI IN TYPE 2 DIABETES MELLITUS SUBJECTS

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Background and Aims: Plasma glucose, plasma insulin and NEFA responses to a food are important determinants for the suitability of that particular item for diabetic patients. Glycemic Index (GI), Insulin Index (II) and NEFA Index (NI) are useful measures for the plasma glucose, insulin and NEFA responses of a dietary component. This study was undertaken to determine those indicators in two of the most popular starchy foods in Bangladesh.

Materials and Methods: Parboiled rice, (BR32 and BR25) and chapati (parboiled, chapati 1 and non-parboiled, chapati 2) were used as test meal and white bread was used as the reference food. Seventeen subjects consumed equi-carbohydrate of the test meal with a run-in period of 5 days between the consecutive items. Plasma glucose was measured by glucose oxidase method, HbA1c and NEFA by colorimetric method and plasma insulin by a fluorimetric microparticle enhanced fluorescent immunoassay (EIMA).

Results: The patients had HbA1c 7.7 ±0.7% (M±SD). Two varieties of chapati lowered the GI values compared to that of bread and pressure parboiled rice (iAUC, Mean±SD, 516±177 in BR32, 531±251 in BR25, 477±235 in Bread vs 399±174 in chapati 1 and 384±164 in chapati 2; BR32, BR25 vs chapati 2, p=0.003; p=0.038). The GI values of rice and chapati were also higher in BR32: 141±96, BR25: 131±73, chapati 1: 116±109 and chapati 2: 105±89; BR32 vs chapati 1, p=0.008. II values were also higher in BR32 (127±87, p=0.033), BR25 (122±71, p=0.019) compared to chapati 2 (92±54). Chapati 1 (101±59) showed lower II than that of BR32 and BR25 though the difference was not significant. NI of BR32 (256±193) was higher compared to that of BR25 (165±107, p=0.040), Chapati 1 (148±108, p=0.042) and Chapati 2 (163±132, p=0.046).

Conclusions: a) Equi-carbohydrate amount of chapati produces lower glycemic responses as compared to Bread and Rice b) Chapati is a better choice than rice from the standpoint of Insulin and NEFA responses c) GI, II and NI are more useful marker in ranking the carbohydrate containing food compared to the absolute blood levels of those parameters.

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RELATION BETWEEN BODY MASS INDEX AND ITS VARIABILITY AND MORTALITY IN TYPE 2 DIABETIC PATIENTS

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The relation between body weight and mortality in type 2 diabetic patients is not clear yet. The aim of the present study was to evaluate the impact of body weight and its variability over time on mortality in a well characterized type 2 diabetic cohort. In the frame of the Verona Diabetes Study, we examined a cohort of 3475 type 2 diabetic patients, whose weight and height were available in 1986 (baseline). Body Mass Index (BMI) variability and its prognostic significance was evaluated in a subgroup of 2039 subjects who had at least two BMI determinations during each of the years 1984, '85, '86. For this purpose, the mean, the coefficient of variation and the slope of BMI was calculated for each individual. During the 10 years of follow-up, 1253 deaths (670 women, 583 men) were identified in the population under study. In the Cox proportional model (adjusted for: age, duration of diabetes, treatment, smoking, hypertension and fasting plasma glucose) analysis leaner women (i.e., those of the first quartile), but not men, showed a significantly lower survival probability than women of third and fourth quartiles. Since the interaction between BMI and age was statistically significant in both sexes, the relation was studied separately in people aged < 65 and > 65 years (median age of the cohort). Under 65 yrs, BMI was directly related to mortality from all causes in both sexes, although the trend achieved the significance only in men (p=0.014). After 65 yrs, higher body weight was associated with a better outcome, especially in women (p=0.012). BMI variability was associated with a significant risk of death, mainly due to cardiovascular diseases, in women and with a similar, although not significant, trend in men. In conclusion, we documented that in type 2 diabetic patients a stable or a moderate excess weight improves survival, while fluctuations of body weight, especially in women, is detrimental to survival.

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Combination Therapy for Glycaemic Control in Type 2 Diabetes

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GLYCEMIC CONTROL IN TYPE 2 DIABETES IS IMPROVED BY LONG-TERM PIOGLITAZONE/SULFONYLUREA COMBINATION THERAPY
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Background and Aims: The durability of glycemic effects of pioglitazone HCl (PIO)/sulfonylurea (SU) combination therapy was studied in patients with type 2 diabetes mellitus. One-hundred twenty-nine patients who completed a long-term, open-label extension (OLE) trial following a 16-week, randomized, double-blind (DB), multicenter trial of PIO in combination with SU vs SU alone were evaluated.

Materials and Methods: On completion of the DB phase, patients entering the OLE from either DB treatment group (ie, PIO/SU; SU alone) started the OLE with PIO 15 mg once daily for 4 weeks, after which the dose could be titrated to 30 or 45 mg/day if needed. The SU dose could be decreased or discontinued, if necessary, in response to hypoglycemia.

Results: Primary glycemic data for a total of 88 weeks are shown*:

	Baseline	End of DB (week 16)	After 24 weeks of OLE (week 40)	After 72 weeks of OLE (week 88)
PIO/SU (n=92) (Total of 88 weeks' combination therapy)				
HbA _{1c} (%)	9.83	8.56 (-1.28)†	8.28 (-1.55)††	8.20 (-1.64)††
FPG (mg/dL)	237.5	180.6 (-56.9)†	174.8 (-62.6)†	171.2 (-66.3)†
SU alone for 16-week DB; SU/PIO for OLE (n=37) (Total of 72 weeks' combination therapy)				
HbA _{1c} (%)	9.27	9.13 (-0.14)	7.97 (-1.29)††	8.02 (-1.25)††
FPG (mg/dL)	222.4	218.6 (-3.9)	163.2 (-59.2)††	164.7 (-57.7)††

*Values reported as Mean (Mean Δ vs Baseline); † p<0.01 vs baseline; †† p<0.01 vs end of DB

Patients who had received combination SU/PIO therapy during the DB phase demonstrated continued improvements in HbA_{1c} and sustained improvements in FPG throughout the 72-week OLE. Patients who had received only SU during DB treatment showed significant improvements in glycemic control when PIO was added; these changes were evident by 24 weeks and were maintained throughout the OLE.

Conclusions: Long-term combination therapy with PIO/SU is an effective treatment option for improving glycemic control in patients with type 2 diabetes and is associated with sustained improvements over time.

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LONG-TERM PIOGLITAZONE/INSULIN COMBINATION THERAPY IMPROVES GLYCEMIC CONTROL IN TYPE 2 DIABETES

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Background and Aims: The durability of glycemic effects of pioglitazone HCl (PIO)/insulin (INS) combination therapy was studied in patients with type 2 diabetes mellitus. One-hundred fifty patients who completed a long-term, open-label extension (OLE) trial following a 16-week, randomized, double-blind (DB), multicenter trial of PIO in combination with INS versus INS alone were evaluated.

Materials and Methods: On completion of the DB period, patients entering the OLE from either DB treatment group (ie, PIO/INS; INS alone) started the OLE with PIO 15 mg once daily for 4 weeks, after which the dose could be titrated to 30 or 45 mg/day if needed. The INS dose could be decreased, if necessary, in response to hypoglycemia.

Results: Primary glycemic data for a total of 88 weeks are shown below*:

	Baseline	End of DB (week 16)	After 24 weeks of OLE (week 40)	After 72 weeks of OLE (week 88)
PIO/INS (n=104) (Total of 88 weeks' combination therapy)				
HbA _{1c} (%)	9.56	8.38 (-1.18)†	8.32 (-1.22)†	8.26 (-1.30)†
FPG (mg/dL)	212.2	173.4 (-38.7)†	160.3 (-51.0)†	152.9 (-59.3)††
INS alone for 16-week DB; INS/PIO for OLE (n=46) (Total of 72 weeks' combination therapy)				
HbA _{1c} (%)	9.43	9.17 (-0.26)	8.21 (-1.22)††	8.26 (-1.17)††
FPG (mg/dL)	217.8	193.9 (-24.0)	166.2 (-51.6)†	169.5 (-48.4)†

*Values reported as Mean (Mean Δ vs Baseline); † p<0.01 vs baseline; †† p<0.01 vs end of DB

Patients who had received combination PIO/INS therapy during DB treatment showed sustained improvements in glycemic control throughout the 72-week OLE. Patients who had received INS alone during DB treatment showed significant improvements in glycemic control when PIO therapy was added; these improvements were evident by 24 weeks and were maintained throughout the OLE.

Conclusions: Long-term pioglitazone/insulin combination therapy is effective in providing sustained control of HbA_{1c} and FPG.

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ROSIGLITAZONE PLUS GLICLAZIDE IMPROVES GLYCAEMIA IN TYPE 2 DIABETICS COMPARED TO DOUBLING THE GLICLAZIDE DOSE

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Background and Aims: This study evaluated the effect of rosiglitazone (RSG) plus half-maximal dose of gliclazide (GLIC) in reducing HbA_{1c} in Type 2 diabetes compared with the up-titration of GLIC alone. **Materials and Methods:** In a 26-week study, patients were randomly assigned to receive either RSG 4 mg twice daily plus GLIC 160 mg/day, or GLIC up-titrated to a maximum of 320 mg/day. At baseline, all patients were receiving GLIC 160 mg/day and had equivalent fasting plasma glucose (FPG) values. **Results:** RSG plus GLIC produced significantly greater decreases in HbA_{1c} and FPG than up-titration of GLIC and was safe and well tolerated. The number of patients with hypoglycaemia and severe oedema was low (RSG+GLIC [n=231] 6.1% and 0.9% respectively; GLIC [n=242] 2.1% and 0.0% respectively). **Conclusions:** 8 mg/day RSG is safe, well tolerated and significantly more effective when added to a half-maximal dose of GLIC than up-titrating GLIC to a maximum dose in improving glycaemia in Type 2 diabetes.

	Treatment group	
	RSG + GLIC	Up-titrated GLIC
HbA _{1c} (%)	n=218	n=233
Mean baseline	8.53	8.59
Mean Δ from baseline ± SD	-1.22* ± 1.100	+0.07 ± 0.906
Difference from up-titrated GLIC	-1.32**	NA
% with reduction ≥ 0.7%	64.7%	20.6%
FPG (mg/dl)	n=225	n=241
Mean baseline	185.1	184.2
Mean Δ from baseline ± SD	-43.4* ± 43.06	+9.2 ± 47.48
Difference from up-titrated GLIC	-53.9**	NA
% with reduction ≥ 30 mg/dl	64.4%	18.7%

*p<0.0001, **p=0.0001

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IN PATIENTS WITH TYPE 2 DIABETES ON METFORMIN, PIOGLITAZONE REDUCES THE ATHEROGENIC INDEX OF PLASMA

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Background and Aims: The Atherogenic Index of Plasma (AIP) is Log (Tg/HDL-C) and correlates inversely with LDL particle size (r = -0.78). Cohorts at low risk for coronary heart disease (CHD) have AIP values <0; those with high risk have positive values. People with type 2 diabetes mellitus (T2DM) are at high risk for CHD and have higher AIPs than their matched controls. We studied the effect of pioglitazone (PIO), a thiazolidinedione, on AIP in patients with T2DM currently treated with metformin (MET) and not in good glycemic control (HbA_{1c} ≥ 8.0%).

Materials and Methods: The AIPs of patients with T2DM who participated in a multicenter, double-blind clinical trial were calculated and compared for treatment effects. After a 6-week run-in period, patients were randomized to either placebo with MET (n=143) or PIO 30 mg QD with MET (n=158). Patients remained on the assigned treatment for the next 16 weeks. Fasting blood samples were obtained for HbA_{1c}, plasma cholesterol (total-C, LDL-C, and HDL-C), Tg, glucose, and insulin. The least-squares mean change (Δ) in lipids, HbA_{1c}, and AIP from baseline (last observation carried forward) for each group was obtained from analysis of covariance model with treatment, center and baseline included as a covariate.

Results: Compared with placebo, PIO lowered HbA_{1c} by 0.83% points and Tg by 16%, raised HDL-C by 8.7%, and had no significant effect on total-C or LDL-C. The results for AIP are shown below:

	Baseline AIP	Mean Δ AIP	SEM	P-value vs Baseline	P-value vs placebo
Placebo + MET	0.36	0.0	0.017	P=0.93	
PIO + MET	0.37	-0.09	0.016	P=0.0001	P=0.0001

Conclusions: Pioglitazone added to metformin significantly reduced AIP in patients with T2DM. Because of the inverse relationship between AIP and LDL particle size, this decrease in AIP may be extrapolated to reflect an increase in LDL particle size.

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GLYCAEMIC EFFICACY AND WEIGHT REGULATION IN PATIENTS ON SULPHONYLUREAS: A UTILITY FUNCTION TO QUANTIFY OUTCOME

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Background and Aims: Body mass index does not predict adverse cardiovascular outcome, but weight gain during therapy (e.g. with sulphonylureas) may impair quality of life. Sulphonylureas, however, remain effective and necessary therapy for many patients. Distinguishing the best overall patient outcome between therapies can be difficult where HbA1c decreases and weight may increase. We present a novel utility function (Wh) that identifies the best overall patient outcome in such situations.

Materials and Methods: Data were analysed from a 20-week, double-blind trial in 806 diet-failed patients randomised to receive fixed combinations of metformin-glibenclamide (M-G) 250mg/1.25mg or 500mg/2.5mg, glibenclamide (G) 2.5mg, metformin (M) 500mg, or placebo. Wh is defined as follows:

$$Wh = [(final\ HbA1c / baseline\ HbA1c) \times (final\ weight / baseline\ weight) - 1]$$

Effects of decreases in HbA1c (efficacy) on Wh are opposed by increases in weight, so that larger negative Wh values suggest better treatment, overall.

Results: Wh was distributed normally (overall mean -0.14, SD 0.12). One-way analysis and multiple comparison t-Test of Student-Newman-Keuls revealed three different categories of treatment, corresponding to the two M-G combination groups (mean Wh = -0.18 for each), the G and M monotherapy groups (Wh = -0.14 and -0.15), and placebo (Wh = -0.05). Multiple regression showed that effects on Wh of M-G 250mg/1.25mg and 500mg/2.5mg were generally superior to those of G (p=0.038 and p=0.005) and M (p=0.066 and p=0.011). Age, gender, initial BMI, duration of diabetes or race did not have a clinically significant influence on Wh.

Conclusions: The novel composite utility function, Wh, shows that combination therapy with M-G is associated with a better overall balance between control of HbA1c and weight gain than monotherapy with either M or G.

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MANAGING THE TOTAL GLYCAEMIC BURDEN IN TYPE 2 DIABETES WITH METFORMIN-GLIBENCLAMIDE COMBINATION THERAPY

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Background and Aims: Tight control of HbA1c has been linked to a reduced risk of diabetic complications including mortality. Elevated fasting and 2-hour postprandial plasma glucose (FPG and 2hPPG) contribute to the total glycaemic burden. A double-blind trial in diet-failed patients has evaluated the effects of glibenclamide (G) and metformin (M), alone, and in fixed combination (M-G), on FPG and 2hPPG.

Materials and Methods: Patients (n=806) were randomised to G 2.5mg, M 500mg, M-G 250mg/1.25mg or 500mg/2.5mg, or placebo (P) for 20 weeks.

Results: All treatments reduced FPG and 2hPPG vs. placebo (Table 1). M-G 250mg/1.25mg and 500mg/2.5mg reduced FPG more effectively than M (treatment differences -1.1 [95%CI -1.5,-0.7] and -1.0 [-1.4,-0.7] mmol/l) and reduced 2hPPG significantly vs. M (-1.1 [-1.8,-0.5] and -1.0 [-1.7,-0.3] mmol/l) and G (-1.0 [-1.7,-0.4] and -0.9 [-1.6,-0.2] mmol/l). The magnitudes of glucose excursions (2hPPG-FPG) were reduced significantly by M and M-G. Effects at 20 weeks on FPG and 2hPPG were larger in patients with more severe diabetes (HbA1c ≥ 8%) at baseline.

Table 1. Mean changes from baseline (mmol/l, means [SE])

	P	G 2.5mg	M 500 mg	M-G 250mg/1.25mg	M-G 500mg/2.5mg
FPG	0.3 (0.1)	-2.0 (0.1)*	-1.2 (0.1)*	-2.3 (0.1)*†	-2.2 (0.1)*†
2hPPG	0.3 (0.3)	-2.4 (0.2)*	-2.2 (0.2)*	-3.4 (0.2)*†	-3.3 (0.2)*†
PPG-FPG	0.0 (0.2)	-0.4 (0.3)	-0.8 (0.2)*	-1.2 (0.2)*	-1.0 (0.2)*

Significantly (p<0.05-0.001) more effective than *P, †M, ‡G.

Conclusions: M-G combinations reduced FPG more effectively than M, and 2hPPG more effectively than M or G. M-G combinations alleviated the total glycaemic burden more effectively than monotherapy with M or G.

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Comparison of glimepiride & gliclazide in metformin-treated patients with type 2 diabetes: a double-blind, crossover study of vascular function.

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Background & Aims: Sulphonylureas (SUs) have differential binding properties to cardiovascular K_{ATP} channels, but the comparative effects of Glimepiride (GLIM) and Gliclazide (GLIC) on micro- and macrovascular function have not been previously reported in patients with type 2 diabetes (T2DM).

Method: Twelve patients with T2DM and suboptimal glycaemic control despite metformin 500mg bid participated in a double-blind, randomised crossover study of additional GLIM 2mg od and GLIC 80mg bid, each for 4 wks, with a 4-wk washout.

Results: Metformin continued unchanged, and patients attended 4 study days after 1st dose and 4-wks administration of each SU to assess metabolic changes (Fructosamine, lipids), arterial stiffness (Augmentation index [AI₁], pulse wave analysis), microvascular reactivity (Peak laser doppler responses to cutaneous iontophoresis of acetylcholine [Ach] & nitroprusside [SNP]) and pressor responsiveness to iv infusions of angiotensin II (AII) and norrenaline (NA).

Parameters	GLIM		GLIC		Diff±SE	p
	1 st dose	1 mth	1 st dose	1 mth		
Fructosamine	329.6	313.7	330.5	318.5	11.8±24	0.6
AI ₁	9.8	9.1	10	9.8	1.2±4.7	0.8
Laser (Ach)	55.7	78.54	44.8	66.8	-1.8±41	0.96
Laser (SNP)	96.8	82.1	101.9	109.5	45.7±87	0.6

Conclusion: Despite experimental evidence of different K_{ATP} binding profiles, there were no clinically significant differences between GLIM & GLIC in terms of metabolic control, and micro- or macrovascular vasodilator and vasoconstrictor reactivity.

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THE COMBINED METFORMIN & SULFONYLUREA THERAPY DOES NOT INCREASE THE RISK OF DEATH IN TYPE 2 DIABETES MELLITUS

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Background: Evidence has been presented suggesting a higher mortality risk brought about by SU (sulphonylurea) and MF (metformin) in combination in type 2 diabetes. **Materials & methods:** Among 4419 type 2 diabetic patients with the disease of up to 10 years' duration, the final therapy had been prescribed prior to the onset of the long follow-up: insulin alone; insulin+oral agents; SU only; BI (biguanides)+SU; BI only; and diet alone. After 26-years' follow-up (1973/74-1999) 3754 deaths were ascertained. Cox multiple regression models were used in assessing the relative odds of deaths from: all causes, coronary heart disease (CHD), and cerebrovascular disease (CVD). Different therapies were represented by a set of dummy variables, with SU therapy used as standard. **Results:** In the Table relative odds are presented for other adjusted risk variables, and 95% confidence intervals.

Cause/treatment	Insulin only	Insulin+oral ag.	SU+BI	BI only	Diet only
All causes	1.43 [1.2-1.6]	1.31 [1.1-1.6]	1.00 [0.9-1.1]	0.72 [0.6-0.8]	0.60 [0.5-0.7]
CHD	1.67 [1.2-2.3]	1.85 [1.2-2.8]	1.10 [0.9-1.3]	0.71 [0.5-1.0]	0.51 [0.4-0.7]
CVD	1.81 [1.3-2.6]	2.70 [1.7-4.4]	0.91 [0.7-1.2]	0.57 [0.4-0.9]	0.34 [0.2-0.5]

Conclusion: The choice of insulin, or insulin & oral agent as the preferred treatment in the first decade of duration of type 2 diabetes predicted an excess death rate during the long follow-up period compared with SU alone. The preference for BI or diet produced lower mortality risk, whereas the combination of oral agents brought the results equivalent to that of single SU treatment.

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REPAGLINIDE PLUS METFORMIN: THERAPY EFFECTS ON INSULIN SECRETION AND SENSITIVITY IN TYPE 2 DIABETES.N. Roudovitch, M. Leyck Dieken² and A.F.H. Pfeiffer¹.¹Clinic B. Franklin, Free University of Berlin and DIFE, Potsdam; ²Novo Nordisk Pharma GmbH, Mainz, Germany.

Background and Aims: Repaglinide (REP) is a novel rapid-acting oral anti-diabetic agent. It acts directly on the pancreatic β -cells to stimulate insulin secretion. The primary objective of this study was to evaluate the hypoglycemic potential of REP under hyper- and euglycemic clamp conditions. Secondly, assess the impact of replacing/restoring early glucose dependent insulin release. Thirdly, the evaluation the effects of REP in combination with metformin (MET) on insulin sensitivity.

Materials and Methods: We quantified β -cell secretion and insulin sensitivity following hyperinsulinemic, euglycemic (EC) (at 4.5 mmol/l) and hyperglycemic clamp (HC) (at 11.1 mmol/l) in 11 patients with T2DM (10M/F; age 58.8 \pm 9 yr., x \pm SE; BMI 32.2 \pm 6 kg/m²; HbA1c 7.6 \pm 1 %; diabetes duration 8.2 \pm 5 yr.) in randomised placebo-controlled study of 2 weeks duration. Two doses of REP 1 mg or PL were given in random order at -30 and 60 min of the clamp. β -cell secretion was determined in the HC as the acute insulin response to glucose (AIRglu) over first 10 minutes and second phase of insulin secretion (I HC 25-180). Insulin sensitivity index (ISI) was measured during the last 30 min of the EC. C-peptide as a marker of insulin secretion in euglycemia was measured under basal and under steady-state conditions. Values are listed as the mean \pm SEM. Statistical significance was determined using paired t-test and/or Wilcoxon-test.

Results: The AIRglu and second phase of insulin secretion under hyperglycemic conditions were significantly increased in HC with REP (AIRglu 1.23 \pm 0.4 vs. 0.73 \pm 0.4 h⁻¹mU/L, p<0.01 and I HC (25-180) 95.8 \pm 25.7 vs. 52 \pm 16.5 h⁻¹mU/L, p<0.01). The C-peptide concentrations under steady-state conditions were lower in EC with PL (501 \pm 41 vs. 945 \pm 77 pmol/L, p<0.05). The basal C-peptide concentrations in EC with REP were significantly higher compared to C-peptide concentrations in steady-state (127.2 \pm 122 vs. 945 \pm 77 pmol/L, p<0.05). Insulin sensitivity index was increased after 1 week combinations therapy: REP plus PL compared to ISI after 1 week therapy: PL plus MET (6.67 \pm 1.8 vs. 4.96 \pm 1.3 mg per kg⁻¹min⁻¹mU, p<0.05).

Conclusions: REP increased first phase and stimulated second phase insulin responses under hyperglycemic conditions, without markedly enhancing insulin secretion in euglycemia. REP improved the early phases of insulin secretion in subjects with T2DM. REP plus MET combination therapy produced a significant enhancement of ISI compared to MET alone, suggesting a synergistic effect on insulin sensitivity.

903

Long-Term Efficacy of Triple Oral Therapy for Type 2 Diabetes Mellitus

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Background and Aims: We previously reported the efficacy of the addition of a thiazolidinedione (initially troglitazone, later rosiglitazone) to the regimen of patients failing metformin and sulfonylurea therapy. We now report the status of these patients three years after the initiation of triple oral therapy

Materials, Methods and Results: At a mean follow up of 37 months (range 18 to 45), 26 (74.3%) of 35 patients remained well controlled on triple therapy with an average HbA1c of 6.9 \pm 0.3% (upper limit of normal 6.5%); the 9 patients who failed did so after a mean duration of therapy of 30 months (range 18 to 42) with an average HbA1c of 8.8 \pm 0.5%. Both groups gained similar amounts of weight during the study period (14.4 \pm 2.1 vs. 11.6 \pm 3.8 lbs, p=0.54). A search for potential predictors of success and/or failure revealed that both groups had similar baseline characteristics including, age, duration of diabetes, weight, BMI, HbA1c and baseline stimulated C-Peptide, and none of these demonstrated a significant correlation with the response to therapy. The only significant difference found between these groups was a significant increase in the stimulated C-Peptide levels during follow up in the group that remain controlled on triple oral therapy (from 3.6 \pm 0.9 to 5.2 \pm 1.1 ng/ml, p=0.002), compared to no significant change during follow up in the group that failed (from 3.7 \pm 0.8 to 4.2 \pm 0.4 ng/ml, p=0.46).

Conclusions: Triple oral therapy is therefore a viable long-term therapy for a significant proportion of those patients who achieve initial control, particularly those who demonstrate improvement of their pancreatic insulin secretory capacity after the addition of a thiazolidinedione.

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ROSIGLITAZONE IN COMBINATION WITH GLIBENCLAMIDE PLUS METFORMIN IS EFFECTIVE AND WELL TOLERATED IN TYPE 2 DIABETES PATIENTSN. Jones¹, T. Jones¹, L. Menci², J. Xu², M. Freed¹ and M. Kreider². SmithKline Beecham, ¹Harlow, UK, ²Collegeville, PA, USA.

Background and Aims: Owing to the progressive nature of Type 2 diabetes (T2D) and the proven benefits of tight glycaemic control, the need for treatment with two or more anti-diabetic agents is increasing. Rosiglitazone (RSG), a potent PPAR γ agonist, has been shown to produce significant dose-dependent reductions in HbA_{1c} and fasting plasma glucose (FPG) in combination with metformin (MET) or sulphonylureas (SU) compared with baseline or comparator alone. This study evaluated the efficacy and tolerability of RSG as triple therapy in T2D patients inadequately controlled on maximum doses of SU + MET.

Materials and Methods: Patients on at least half-maximal doses of glyburide (GLB) and MET were titrated to maximal doses of both agents (20 mg/day GLB + 2 g/day MET) and entered into a 4-week single-blind, placebo (PBO) run-in period. A total of 837 patients with FPG \geq 140 mg/dl and \leq 270 mg/dl were randomised to either PBO, RSG 4 mg/day or RSG 8 mg/day, in addition to maximum doses of GLB+MET, for 26 weeks.

Results: FPG and HbA_{1c} were approximately 11.2 mmol/l and 8.7%, respectively, at baseline. Following 26 weeks' treatment, significant reductions in mean HbA_{1c} and FPG from PBO were observed for RSG 4 mg/day (-0.58%, -1.7 mmol/l) and for RSG 8 mg/day (-1.09%, -2.9 mmol/l). In the RSG 8 mg/day group, 49% of patients achieved an FPG<140 mg/dl, and 63% had a decrease in HbA_{1c} \geq 0.7%. Symptoms of hypoglycaemia, effectively managed by reducing the doses of GLB and/or MET, were more commonly reported in the RSG groups. Mild-to-moderate oedema was also reported more frequently in the RSG treatment groups but infrequently resulted in withdrawal from therapy.

Conclusions: RSG (4 and 8 mg/day) triple combination with GLB + MET was efficacious and well tolerated, producing marked improvement in glycaemic control in patients with T2D previously inadequately controlled on GLB + MET.

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New Glucose-Lowering Possibilities – Human Studies

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ACUTE EFFECTS OF THE DITERPENE GLYCOSIDE STEVIOSIDE IN TYPE II DIABETIC PATIENTS.

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Background and Aims: Extracts of the plant *Stevia rebaudiana* Bertoni (SrB) have been used for many years in the treatment of diabetes in South America, however, without clinical substantiation. We have previously demonstrated that Stevioside, a diterpene glycoside present in the plant SrB, possesses insulinotropic, glucagonostatic and anti-hyperglycaemic potentials in animal studies. The question arises whether Stevioside also in type 2 diabetes exerts beneficial effects on the glucose metabolism. Hypothesis: Stevioside added to a test meal reduces the postprandial blood glucose levels, stimulates insulin and suppresses glucagon levels.

Materials and methods: An acute, paired, cross-over study was carried out in twelve type 2 diabetic subjects (4 females/8 males) with a mean glycated hemoglobin A1C of 7.4±0.4%. A standard meal (1725 kJ) was supplemented with either 1 g of Stevioside or 1 g of gelatine (placebo). Blood samples were drawn from an antecubital vein 30 minutes before and 240 minutes after ingestion of the test meal. Students paired t-test was used for comparing the effects of stevioside with placebo on the parameters measured.

Results: Compared to placebo, stevioside reduced the incremental area under the glucose response curve with 18% ($p=0.013$), the area under the glucagon response curve by 19% ($p=0.02$), and the glucagon like peptide-1 (GLP-1) response by 31% ($p=0.044$). Stevioside did not significantly alter the area under the insulin and glucose-dependent insulinotropic polypeptide (GIP) curves and no differences in the postprandial levels of free fatty acids (FFA) and triglycerides were found.

Conclusions: Stevioside reduces postprandial blood glucose and glucagon levels in type II diabetic patients. This indicates that Stevioside has beneficial effects on the glucose metabolism and may be advantageous in the treatment of type II diabetes.

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6 MONTH EFFICACY AND SAFETY OF BENFLUOREX vs. PLACEBO AND METFORMIN IN TYPE 2 DIABETIC PATIENTS.

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Aims: 6-month efficacy of Benfluorex (Mediator^{*}) (150–450 mg/day) was assessed in a double-blind multicentre study vs. Placebo and vs. Metformin (850–2550 mg/day). **Materials and methods:** After a 2-month run-in period of strict dieting, 722 type 2 diabetic patients were randomized (1:2:2) to Placebo (PI; n=144), Benfluorex (BF; n=294) or Metformin (MF; n=284). After a 5-week dose-finding phase, the efficacy of BF was compared with PI (test for difference, main analysis) and MF (non-inferiority test, secondary analysis) during a 6-month fixed dose treatment. **Results:** At entry after strict dieting there was no difference for HbA_{1c} (PI: 7.4±1.5, BF: 7.7±1.6, MF: 7.8±1.6%) and fasting plasma glucose (FPG; PI: 9.7±2.3; BF: 10.0±2.0; MF: 10.2±2.5 mmol/l). At the end of the dose-finding phase, mean dose was PI: 2.71 tab/d; BF: 2.65 tab/d (397.5 mg/d); MF: 2.50 tab/d (2125 mg/d). At the end of treatment (ITT), HbA_{1c} level decreased by 0.60% ($p < 0.001$) in BF patients and increased by 0.50% ($p < 0.001$) with PI, mean endpoint difference -0.86 (0.17)% ($p < 0.001$). FPG decreased by 1.24±2.30 mmol/l with BF and increased by 0.36±2.73 mmol/l with PI; mean endpoint difference -1.33 (0.28) mmol/l ($p < 0.001$). In patients treated with MF, HbA_{1c} level decreased by 0.60% ($p < 0.001$); mean endpoint difference vs. BF was 0.28 (0.12)% [90% CI: 0.07; 0.48]. With MF, FPG decreased by 1.24±2.30 mmol/l ($p < 0.001$); mean endpoint difference with BF: 0.64 (0.19) mmol/l [90% CI: 0.33; 0.95]. Fasting plasma insulin, total and HDL-cholesterol, and triglyceride concentrations were not different among treatments. Treatment with BF was well tolerated; 22% of BF patients reported one or more adverse events (PI: 17%; MF: 23%) and only two patients suffered a treatment-related, serious adverse event. **Conclusion:** This study demonstrates that BF 1. significantly reduces HbA_{1c} and FPG when compared to placebo; 2. has a good safety profile; and 3. has relatively lower potency as compared to MF, though a non inferiority test (equivalence limit for HbA_{1c} of 0.5%) was not different.

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Achieving goals metabolic control in type 2 diabetes mellitus by treating postprandial hyperglycaemia with Miglitol.

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Background and Aims: To evaluate the effectivity of Miglitol in glucose control and to identify factors related with the treatment response in a population of type 2 diabetes mellitus (DM) patients.

Materials and Methods: 5.395 out-patients with type 2 DM participated in a prospective, observational and multicentre study performed in Spain. Once included, they could be treated with Miglitol (alone or in combination with other antihyperglycemic drugs) if HbA_{1c} > 6.5% with the intention to achieve blood glucose control ("low risk") according to the European Diabetes Policy Group 98-99 (EDPG98-99): HbA_{1c} ≤ 6.5%, fasting glucose < 110 mg/dl (6.0 mmol/l) and postprandial glucose < 135 mg/dl (7.5 mmol/l). After 12 months of treatment with Miglitol in a dose up to 300 mg/tid, a multivariate logistic regression analysis was performed.

Results: At the inclusion visit 91.5% of patients with type 2 DM showed "microvascular risk" according to EDPG98-99: HbA_{1c} > 7.5%, fasting glucose > 125 mg/dl (7.0 mmol/l) or postprandial glucose > 160 mg/dl (9.0 mmol/l). Recently diagnosed patients (less than 1 year) had an average HbA_{1c} of 7.0% (SD 1.3) in comparison with previously diagnosed patients (more than 1 year) who presented an average HbA_{1c} of 7.6% (SD 1.4). Maximum effectivity of Miglitol was observed in patients with HbA_{1c} > 7.5% at baseline (mean decrease -1.8%, CI 95% -1.67 to 1.86%) and among those practising physical exercise (OR: 1.67; CI 95% 1.15 to 2.42). Multivariate analysis showed that a good response to treatment was not associated with age, gender, smoking habits or hypercholesterolemia at diagnosis. 74% of recently diagnosed patients achieved blood glucose control according to EDPG in comparison to 50% of previously diagnosed ones ($p < 0.001$). Patients treated with Miglitol in monotherapy showed better results in comparison with those treated with combination therapy (66% vs 44%, $p < 0.001$). The percentage of bad controlled patients was also reduced from 91.5% at baseline to 48% at the end of the study. At inclusion visit, 27% of patients had postprandial glucose below 180 mg/dl (10.0 mmol/l) and moved up to 70% after 12 months of treatment with Miglitol.

Conclusions: Miglitol is effective in the treatment of type 2 DM patients, particularly in those with a worse metabolic control who reach good HbA_{1c} and blood glucose values after 12 months of treatment.

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EFFECT OF MIGLITOL ON TYPE 2 DIABETIC PATIENTS

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Background and Aims: Miglitol is a new α -glucosidase inhibitor. Aims of the study :To evaluate the effect of miglitol on glucose and lipid profiles in type 2 diabetics and to asses drug tolerability.

Metirials and Methods: Following a two-week run-in period, 20 Type 2 diabetics, (10 males) with diabetes of at least 6 months duration (or stabilized for at least 3 months), 40-75 years, non obese, with HbA_{1c} > 8% on diet (n=5) or sulphonylureas (n=15) were blindly randomized to receive either placebo or miglitol 50 mg tds, for 4 weeks with a 2 week wash-out period on placebo (double blind, placebo controlled, crossover trial). Adverse event monitoring was done at each visit. **Results:** At the end of the placebo period mean fasting blood glucose=13.4±3.4mmol/l, HbA_{1c}=9.5±2.8%, fructosamine=3.2±0.56mmol/l, total cholesterol=6.59±1.28mmol/l, triglycerides=1.9±1.02 mmol/l, body weight=74.7±14 kg. At the end of the actual therapy mean fasting blood glucose=12.8±3.2mmol/l, HbA_{1c}=9.86±2.8%, fructosamine=3.1±0.56 mmol/l, total cholesterol=6.7±1.2mmol/l, triglycerides=2.02±1.25mmol/l, body weight=74.7±14.4 kg. There was no statistical difference between miglitol and placebo in fasting blood glucose ($p < 0.99$), HbA_{1c} ($p < 0.99$), fructosamine ($p < 1$), total cholesterol ($p < 1$), triglycerides ($p < 0.99$) and weight=74.7±14.4 ($p < 1$). The most frequently adverse events reported were flatulence and meteorism which occurred 55% more frequently in miglitol vs. placebo treated patients ($p < 0.002$). Nobody stopped the drug due to adverse events. **Conclusions:** there are no benefits from this drug but only adverse reactions.

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IGF-1/BP3 (Somatokine) is well tolerated and is biologically active in patients with type 2 diabetes mellitus receiving insulin.

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Background and Aims: Previous studies clearly demonstrated the efficacy of recombinant human insulin-like growth factor I (rhIGF-1) in subjects with type 1 and type 2 diabetes. rhIGF-1 decreased HbA1c in both types of diabetes and reduced insulin requirements in type 1 diabetes. It also increased insulin sensitivity in subjects with type 2 diabetes and in severe insulin resistance. Concerns have been raised about the dose-dependent side effects produced by rhIGF-1. In a short-term, proof of concept study, we previously demonstrated that an equimolar ratio of rhIGF-1 and rhIGFBP-3 (Somatokine) was biologically active in patients with type 1 diabetes.

Materials and Methods: In the current study, subjects with type 2 diabetes were exposed to 4 dosage regimens of rhIGF-1/BP-3 determine pharmacokinetics and pharmacodynamics, clinical effects, and the adverse event profile. Somatokine was administered in one of 4 regimens: 1) twice daily sc at a dose of 2 mg/kg/24 hr; 2) as a continuous sc infusion of 2 mg/kg/24 hr; 3) as a 6 hr overnight sc infusion of total dose of 2 mg/kg/24 hr; and 4) as a single sc injection of 1 mg/kg/24 hr. Endpoints included mean 24 hour blood glucose, MAGE, and 24 hour insulin dose.

Results: Each dose produced a statistically and clinically significant reduction in insulin requirements and improved glycemic control. Importantly, each dose was well-tolerated. There were no drug-related serious adverse events in any study group, despite the fact that the dose of total IGF-1 ranged up to 2.5 times the dose that was poorly tolerated in previous studies of free rhIGF-1. One subject did develop a mild Bell's Palsy which resolved spontaneously. The adverse events reported most frequently included headache (9/12 group 2 and 7/14 group 1) back pain (3/12 group 1 and 7/14 group 2) and vomiting (2/13 group 3 and 2/16 group 4). No subjects discontinued from the study because of adverse events.

Conclusions: These data are consistent with earlier trials of Somatokine in other patient groups without diabetes, but distinctly different than studies with free IGF-1. For Somatokine, there appears to be a dissociation between doses that produce a clinically significant effect from those that produce serious adverse events. Larger scale clinical trials are underway to determine the full dose response of Somatokine in diabetes both for clinical efficacy and tolerance, but the current data suggest that Somatokine may have a significant place in the therapy of diabetes mellitus. The safety profile of Somatokine is superior to that of rhIGF-1 alone.

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Amylin replacement with pramlintide as an adjunct to insulin therapy facilitates a combined improvement in glycemic and weight control in type 2 diabetes.

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Background and Aims: Among the most prominent limitations of insulin therapy in type 2 diabetes are undesired weight gain and failure to achieve satisfactory glycemic control, especially during the postprandial period. Amylin, a beta cell hormone that is co-secreted with insulin in response to nutrient stimuli, reduces food intake in rodents and complements the effects of insulin in postprandial glucose homeostasis by regulating the rate of glucose influx after meals. Postprandial amylin responses are markedly impaired in insulin-treated type 2 diabetic patients. Three randomized-controlled clinical trials have consistently shown that preprandial s.c. injections of pramlintide, a synthetic analogue of human amylin, as an adjunct to insulin therapy result in significant mean reductions in both HbA1c and body weight in patients with type 2 diabetes. To further characterize this dual effect of pramlintide, we conducted a pooled analysis of all three clinical trials.

Materials and Methods: Type 2 diabetic patients treated with either placebo+insulin [n=207, baseline HbA1c 9.3±1.2%, body weight 90.9±19.7kg, (mean±SD)] or pramlintide 120 microgram BID+insulin [n=221, baseline HbA1c 9.1±1.1%, body weight 92.3±20.0kg] were stratified based on the changes (decrease or increase) from baseline in HbA1c and body weight after 26 weeks.

Results: Twice the proportion of patients in the pramlintide+insulin group achieved a reduction in both HbA1c and body weight compared to the placebo+insulin group (51% vs. 26%, p<0.0001). Of the placebo+insulin treated patients, 21% experienced an increase in both HbA1c and weight, compared to only 10% in the pramlintide+insulin group (p<0.0001). Overall, 90% of pramlintide+insulin treated patients achieved a reduction in either HbA1c, weight, or both. Stratification by BMI and occurrence of adverse events revealed that the weight lowering effect of pramlintide was most pronounced in obese patients and unrelated to the occurrence of nausea, the most commonly observed side effect.

Conclusions: The addition of pramlintide to insulin therapy, i.e., the replacement of both amylin and insulin responses at mealtime, facilitates a combined improvement in glycemic and weight control in patients with type 2 diabetes.

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Twelve Weeks or Continuous Administration of GLP-1 in Elderly Diabetic Patients

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Background and Aims: The established insulinotropic effect of GLP-1 had not been confirmed in elderly type 2 diabetes patients for a prolonged period.

Materials and Methods: We examined regulation of glucose homeostasis by GLP-1 (7-36 amide) before and after 12 weeks of continuous subcutaneous administration of GLP-1 (0.1-0.4 nmol/kg/h) in 7 elderly type 2 diabetic patients (age=72.6±1.3, BMI=27.4±1.3). No other agent for regulation of glucose was used in this group. A control group, n=5, was also examined before and after 12 weeks of usual therapy (age=72.2±1.6, BMI=28.0±1.3). Diabetes was diagnosed 14±5 yrs. prior to the study in each group. A hyperglycemic clamp (5.4 mmol/l above fasting) was performed for 1h followed by return to fasting level for 1h, immediately followed by a euglycemic clamp (480 pmol/m2/min) for 2h before and 12 weeks after administration of GLP-1. During the euglycemic clamp, plasma glucose was allowed to fall to 5.3 mmol/l and then maintained. In both groups hypoglycemic agents were withheld for 3 days prior to each clamp, except for GLP-1 in the post clamp.

Results: In the control group 1st phase insulin (IRI) response was absent in the initial and final hyperglycemic clamps and in both clamps, 2nd phase insulin responses were only 50 pmol/l above fasting levels. In euglycemic clamps, 180-240 min IRI levels were 1700 pmol/l in all clamps. In the GLP-1 treated group, 1st phase IRI response was absent in the pre-clamp and improved in the post-clamp while second phase IRI levels increased by 85±36 pmol/l from the first clamp. In 4 individuals who received GLP-1 treatment, we obtained 2-min samples during each of the hyperglycemic clamps and insulin pulse profiles were analyzed with multiparameter deconvolution technique. Insulin burst amplitude increased from 9.4 ±1 to normal values of 31.2±2 pmol/l/min. Fasting plasma glucagon levels (IRG) were lower in the GLP-1 group after 12 weeks but not in the control group (17 v 24 pmol/l). IRG levels during the last h of the clamp were 14 v 18 pmol/l. M values decreased in the post hyperglycemic clamp in the diabetic control group and slightly increased in the GLP-1 group (both NS). During euglycemia a similar change in M was observed.

Conclusions: We conclude that GLP-1 is well tolerated for a prolonged period in elderly type 2 diabetic patients and is as efficacious as the usual oral agents.

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Amylin replacement with pramlintide as an adjunct to insulin therapy facilitates a combined improvement in glycemic and weight control in type 1 diabetes.

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Background and Aims: Type 1 diabetes is characterized by an absolute deficiency of two beta cell hormones, insulin and amylin. Attempts to restore near-normoglycemia with insulin replacement alone fail in the majority of patients, and are accompanied by an increased risk of hypoglycemia and undesired weight gain (DCCT). Amylin reduces food intake in rodents and complements the effects of insulin in postprandial glucose homeostasis by regulating the rate of glucose influx after meals. Three long-term clinical trials in patients with type 1 diabetes have consistently shown that preprandial s.c. injections of pramlintide, a synthetic analogue of human amylin, as an adjunct to insulin therapy result in significant mean reductions in both HbA1c and body weight in patients with type 1 diabetes. To further characterize this dual effect of pramlintide, we conducted a pooled analysis of all three clinical trials.

Materials and Methods: Type 1 diabetic patients treated with placebo+insulin [n=393, baseline HbA1c 8.9±1.2%, body weight 75.7±14.3kg, (mean±SD)] or pramlintide (30/60 microgram TID/QID)+insulin [n=496, baseline HbA1c 8.9±1.2%, body weight 77.0±14.7kg] were stratified based on the changes (decrease or increase) from baseline in HbA1c and body weight after 26 weeks.

Results: Twice the proportion of patients in the pramlintide+insulin group achieved a reduction in both HbA1c and body weight compared to the placebo+insulin group (44% vs. 22%, p<0.0001). While 27% of the placebo+insulin treated patients experienced an increase in both HbA1c and weight, this proportion was less than 9% in the pramlintide+insulin group (p<0.0001). Thus, more than 90% of pramlintide+insulin treated patients achieved a reduction in either HbA1c, weight, or both. Stratification by BMI and occurrence of adverse events revealed that the weight lowering effect of pramlintide was most pronounced in obese patients and unrelated to the occurrence of nausea, the most commonly observed side effect.

Conclusions: The addition of pramlintide to insulin therapy, i.e., the replacement of both missing beta cell hormones at mealtime, facilitates a combined improvement in glycemic and weight control in patients with type 1 diabetes.

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Searching for the Artificial Beta-Cell

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GLYCATED HAEMOGLOBIN AND CONTINUOUS GLUCOSE MONITORING.

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Background. Glycated haemoglobin (HbA_{1c}) is the standard by which glycaemic control of diabetes is assessed. Previous studies have examined the correlations between various markers of glucose control and HbA_{1c}, but new technology allows better assessment of this issue. **Patients and Methods.** 19 patients with insulin treated diabetes (11F, 8M, mean age 41.2 years, range 20 - 77 years) were studied with the continuous glucose monitoring system (MiniMed Inc.). This system measures subcutaneous glucose levels at 5 minute intervals throughout the study period, which levels correlate closely with capillary glucose. **Results.** During a mean study period of 2.7 days per patient (range 1.5-4.2), HbA_{1c} correlated with median glucose level ($r=0.53$, $p=0.02$). Peak glucose, quantitated as time (hours/24 hours) during which the glucose level was above an arbitrary figure of 15mmol/l, also correlated with HbA_{1c} ($r=0.49$, $p=0.035$). Variability in glucose, measured as the interquartile range for measurements, did not correlate with HbA_{1c} ($r=0.02$, $p=0.9$). Neither was there a correlation between any isolated blood glucose value and HbA_{1c} (fasting glucose $r=0.23$, $p=0.35$), (2 hour postprandial glucose, $r=-0.14$, $p=0.56$). **Conclusion.** HbA_{1c} correlates with median and peak glucose levels, but says little about variability in glucose values or any individual level. This fundamental point should be taken into account when using HbA_{1c} to assess effect of therapeutic agents or adjusting insulin regimens.

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Continuous glucose monitor system in children with diabetes mellitus type 1
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Background and Aims. Continuous glucose monitor system is a progressive method in diabetes care. The authors summarized their first experience with continuous glucose monitor system in children.

Materials and Methods: 14 children (7boys, 7 girls) aged from 4 to 13 years were examined by means of continuous glucose monitor system MiniMed. 13 patients suffered from diabetes mellitus type 1 with unstable control, 1 non-diabetic girl was suspicious of fasting hypoglycemia. Monitor was applied with the previous informed consent of the patient's parents. Glucose monitor sensor was introduced into the right or left upper abdominal quadrant subcutaneously and the system was initialized within the next one hour. Glycemic values were then registered every 5 minutes by monitor system without the possibility of patient's visual control and after the maximum of 72 hours displayed on PC equipped with a special software.

Results: Monitoring was successful in 11 children from the total 14. 1 boy refused the monitor after its initialization. In 2 patients small bleeding in the site of sensor application was observed and consequently discontinued monitoring due to blood coagulum in sensor cannula. Successful measurements proved nocturnal hypoglycemia in 5 diabetic children. The remaining 5 diabetic patients had high glycemia at night and in the morning but hypoglycemia in the afternoon. In 1 non-diabetic girl hypoglycemia after fasting was found out at night. All performed and successful monitorings contributed to the insulin dosage modification in diabetic patients and stabilized glycemia. In 1 non-diabetic girl with fasting hypoglycemia the diet containing maltodextrine was applied for hypoglycemia prevention.

Conclusions: Continuous glucose monitor system has contributed to the adequate insulin dosage and to the improved diabetic control. It could be also used for the diagnostics of hypoglycemia of different causes.

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INITIAL EXPERIENCE WITH THE CONTINUOUS GLUCOSE MONITORING SYSTEM

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Background and Aims: Relevant prospective studies (DCCT, UKPDS) have demonstrated that a good metabolic control of DM decreases the risk of complications. Sometimes, intensive insulin therapy plus frequent self-monitoring of blood glucose are not enough to improve glycemic control. The MiniMed® Continuous Glucose Monitoring System (CGMS) offers more detailed information of glucose profile. The aim of the study was to evaluate benefits and problems related with the use of CGMS.

Materials and Methods: 11 patients with badly controlled DM and/or frequent hypoglycemia (glycated hemoglobin $8.23 \pm 1.6\%$) were monitored with the CGMS for 3 days (5 ♂ and 6 ♀; 5 type 1 and 6 type 2; age 53.5 ± 15 years; DM evolution 16.7 ± 9 years). All subjects were treated with insulin (9 with multiple injections and 2 with insulin pump). All CGMS were installed by the same instructed nurse following the recommendations of the system. Patients were instructed adequately and encouraged to maintain their usual lifestyle and treatment, and they used the same glucometer during the monitoring period.

Results: CGMS register duration was 66 ± 7 hours. Technical problems observed were: 1) In 1 case we needed to replace the CGMS 1 hour after it was installed due to an "error" message; 2) in another case an alarm was started due to a big discrepancy between meter glucose levels and sensor glucose levels; 3) and in 3 cases the CGMS interrupted the register for 1 to 5 hours without any apparent reason or alarm. Non-optimal correlations between meter glucose/sensor glucose were observed in all patients in 1 or more days of the monitoring period. We observed 2 especially relevant discrepancies: 1) "False negative of hypoglycemia": clinical hypoglycemia confirmed by the meter not detected by the CGMS; 2) And, "false positive of hypoglycemia": normal meter value whereas sensor value was <40 mg/dl in the absence of symptoms of hypoglycemia. The CGMS was comfortable and not painful, and patients felt confident and satisfied with its use. Only 1 patient showed difficulties in understanding the CGMS. Glucose profile obtained was very informative and useful to modify treatment adequately.

Conclusions: The CGMS is a useful tool for modifying treatment of patients with DM, but the number of technical problems observed needs to be considered.

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AMBULATORY CONTINUOUS SUBCUTANEOUS GLUCOSE SENSOR IN ADOLESCENT WITH TYPE 1 DIABETES: A BENEFICIAL TOOL.

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Background: Puberty is commonly associated with a deterioration of metabolic control in adolescence with type 1 diabetes for physical and psychological reasons. Capillary glucose monitoring is rarely performed and not sufficient to evaluate glycemic control. Hospitalization is often rejected and then medical follow-up difficult in these young patients. **Aim of the study:** To examine the help provided by ambulatory continuous SC glucose monitoring for the care of diabetic adolescents during their usual lifestyle. **Patients:** Eligibility criteria include adolescent with type 1 insulin-dependent diabetes and HbA_{1c} greater than 8%. **Methods:** At the beginning of the study (M0), SC glucose sensor (CGMS, Mimimed, Sylmar, USA) is used for 3 days. HbA_{1c} and Low Blood Glucose Index (LBGI) are determined. After download to a computer, data are examined for evaluation of glycemic variations and discussed with the patient. Then insulin treatment is modified. Two months later (M2) the same procedure is used and all studied parameters are re-evaluated. **Results:** Ten adolescents (5 girls, 5 boys) with type 1 IDDM have been included (mean age (\pm SD): 16.7 ± 1.2 years, mean duration of diabetes: 5.1 ± 3.3 years, mean HbA_{1c} during the previous year: $10.2 \pm 0.8\%$). At inclusion, treatment consists in 2 (n=3) or 3 (n=6) daily insulin injections or CSII (n=1). In 9 patients, variations of glycemia are really revealed by CGMS but do not appear with capillary measures. In 7 patients day-to-day reproducibility of glycemic fluctuations is supposed to be related to inappropriate treatment. HbA_{1c} significantly improves from M0 to M2 (10.3 ± 1.1 vs $9.1 \pm 0.9\%$). LBGI does not significantly change (2.2 ± 1.1 vs 3.2 ± 1.4). Modifications of insulin treatment consist in intensification (n=3), change in insulin doses (n=4) or insulin type (n=3). Daily sensor data are not optimal in 40 % of cases. This is related to the patient (50 %) or to the device (50 %). **Conclusion:** CGMS provides the basis for discussion, motivation and adjustment of insulin treatment leading to improvement of metabolic control in ambulatory adolescent patient with type 1 diabetes. It appears then as a very useful tool in these patients.

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ACCURACY OF MINIMED CONTINUOUS GLUCOSE MONITORING SYSTEM DURING HYPOGLYCAEMIA

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Background and Aims: The concept of glucose sensing is an important development for patients with diabetes as it provides more accurate information about excursions above and below target levels. Clinical experience with the MiniMed continuous glucose monitoring system has revealed a surprising level of previously unrecognised hypoglycaemia. Available evidence suggests that subcutaneous glucose levels closely mimic blood glucose levels with a lag time of only a few minutes. However, no studies have been published to show how well the sensor performs in sustained hypoglycaemia and in recovery from hypoglycaemia. The aim of our study was to evaluate the accuracy of the MiniMed subcutaneous glucose sensor during and in recovery from controlled hypoglycaemia.

Materials and Methods: A hyperinsulinaemic glucose clamp (60mU/m²) was used to study 8 healthy volunteers. 2 glucose sensors were inserted the day before the study and calibrated with pinprick measurements. On the day of the study the sensors were calibrated with glucose values from a yellow springs glucose analyser at the start of the study and at the end of the study. One of the sensors was also calibrated during hypoglycaemia. Blood glucose levels were maintained at euglycaemia for the first 60 mins, then decreased to 2.5 mmol/l for 60 mins and finally restored to euglycaemia for a further 60 mins. Arterialised blood taken was analysed for blood glucose every 5 mins. At the end of the study, sensor readings were compared to blood glucose readings.

Results: Seven sensors did not provide usable data. The other 9 sensor profiles showed good agreement with blood glucose levels at each of the three plateau's with a correlation coefficient of 0.79 and mean absolute difference of 7 % (351 paired readings.) Regression analysis - slope = 0.81, intercept = 0.44 mmol/l. The sensor readings did have a tendency to read lower than the actual blood glucose levels: Average readings at (A) Euglycaemia, (B) Hypoglycaemia (C) Euglycaemia - recovery: Sensor readings: (A) 4.20 +/- 0.60, (B) 2.50 +/- 0.38, (C) 3.95 +/- 0.71. Blood glucose: (A) 4.47 +/- 0.27, (B) 2.58 +/- 0.19, (C) 4.48 +/- 0.17. The sensor drop closely matched the drop in blood glucose but the recovery from hypoglycaemia was a little more variable. The time taken for the sensor to achieve a reading > 4mmol/l after hypoglycaemia was delayed by an average of 30 mins (range 0 - 55 mins).

Conclusions: In general the sensor performed well although there was variation in performance between sensors. They proved effective at recognising the onset of hypoglycaemia, but may be over-estimating the degree of and duration of hypoglycaemia.

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Repeated use of continuous glucose monitoring in children and adolescents improved metabolic control without increasing hypoglycaemia.

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Background and Aims: The CGMS sensor (Continuous Glucose Monitoring System, MiniMed, USA) monitors interstitial subcutaneous glucose (scGlucose) levels (2.2-22 mmol/L), which is said to reflect blood glucose, every 10 seconds and records an average value every 5 minutes. The data is downloaded to a computer providing a continuous tracing of blood glucose values. The aim of this study was to evaluate the possible improvements in metabolic control in children and adolescents when using CGMS.

Materials and Methods: 27 diabetic patients aged 12.8 ± 3.6 (5-20) years and with a diabetes duration of 7.0 ± 3.8 (2 - 15) years (mean + SD) were randomized to use CGMS for 3 days every fortnight in a 6 month cross-over study. Half the time CGMS was used in 'blind' mode, i.e. neither the patient nor the diabetes team took part of the results.

Results: The sensors gave valid readings for 2.1 ± 1.0 days. In both groups there was a decrease in HbA1c during the first 3-month period. After cross-over the now open group had a further decrease in HbA1c (caused mostly by a decrease in nighttime scGlucose) while the now 'blind' group leveled off. Altogether there was a significant decrease in HbA1c during the open arm when there was access to the CGMS data from 7.6 to 7.2% (p=0.012) while the decrease during the 'blind' arm was from 7.6 to 7.5% (p=n.s.). There were 1.5 episodes/day of daytime and 0.6 of nighttime high scGlucose (> 15 mmol/L). 26/27 patients experienced daytime low scGlucose (< 3.0 mmol/L, 0.8 episodes/day, duration 58 ± 29 min) and all patients had at least one nighttime episode of low scGlucose (0.4 episodes/night, duration 132 ± 81 min). We found no difference in low scGlucose frequency between the treatment arms.

Conclusions: Using CGMS in the pediatric age group detects wide swings in scGlucose, sometimes with very low values. With repeated use of this device it was possible to lower HbA1c without increasing the frequency of low scGlucose (< 3mmol/l; reflecting hypoglycaemia).

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A RANDOMIZED, CONTROLLED STUDY: USE OF A CONTINUOUS GLUCOSE SENSOR IMPROVES HBA1C LEVELS.

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Background and Aims: Earlier results of nonrandomized pilot studies suggest that continuous glucose monitoring (CGMS, MiniMed Inc.) can be used to guide therapy adjustments and reduce HbA1c levels. To more rigorously evaluate this question, we undertook a randomized, prospective study in patients with poorly controlled Type 1 diabetes.

Materials and Methods: To be eligible, HbA1c levels had to be ≥ 8% despite at least one year of intensive insulin therapy with MDI or CSII, frequent self-monitored blood glucose (SMBG) tests, quarterly follow-up visits with review of meter downloads and education on self-adjusting insulin. Patients were assigned to one of two methods of therapy adjustment. The CGMS group wore a sensor during Week 1 and Week 3, with each use followed by clinician review and therapy adjustments based on glucose profiles, diet logs, and meter downloads. The Meter group had clinician review and therapy adjustments also at Week 1 and Week 3, but using only data obtained from their meter downloads and diet logs. HbA1c measurements were obtained at Baseline, Week 8 and Week 12.

Results: Twenty patients with an average age of 36 years (range, 19 to 61 years) and with 21 ± 9 years history of diabetes, completed the study. The average HbA1c [CGMS vs. Meter] was not different at Baseline [9.1 vs. 8.9, p=0.683]. However, the average HbA1c was significantly lower for the CGMS group at Week 8 [7.9 vs. 8.6, p=0.064] and Week 12 [7.9 vs. 8.8, p=0.026]. There was a trend towards greater reduction from baseline in HbA1c at Week 8 [-1.2% vs. -0.3%] and Week 12 [-1.2% vs. -0.1%] when therapy adjustments were based on continuous glucose data versus when they were based on meter data alone.

Conclusions: These results confirm that the CGMS is an effective means of reducing HbA1c, especially in patients who have failed to achieve control despite frequent blood glucose monitoring.

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Gestational Diabetes

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Variables related to insulin sensitivity during human non-diabetic pregnancy. M.C. Breschi, G. Seghieri, R. Anichini, A. De Bellis, F. Franconi, and L. Alvisi. Dept. of Internal Medicine and Diabetes Unit, Presidio Ospedaliero USL n.3, Pistoia, and University of Sassari, Italy.

Background and Aims: Insulin resistance is raised during the last trimester of pregnancy. Aim of this study was to estimate the set of clinical variables mostly associated with the 'physiologic' insulin resistance related to the pregnant status.

Materials and Methods: The study group was composed of 436 women who were classified as nondiabetic after having been tested by means of a 100-g OGTT between the 24th and the 28th gestational week, as suggested by the ADA clinical practice recommendations (Diabetes Care 23 Suppl.1:S77-79,2000). Insulin sensitivity was measured using the index of Matsuda and DeFronzo (10,000/square root of [fasting glucose x fasting insulin] x [mean glucose x mean insulin during OGTT]), and validated for pregnancy by Kirwan JP (Diabetes 49 Suppl. 1:A3,2000).

Results: By univariate analysis the insulin sensitivity index (ISI) was inversely related to both pregestational ($r=-0.27$; $p<0.0001$) and actual body mass index ($r=-0.27$; $p<0.0001$), week of test ($r=-0.10$; $p=0.03$), as well as to mean blood pressure ($r=-0.16$; $p=0.0008$), while no significant relationship was present with age, parity or family history of diabetes. Gestational week of test, body mass index (BMI) at onset of pregnancy and mean blood pressure remained significantly related to ISI after a multiple regression analysis model including as covariates age, parity and body weight increase ($p<0.05$).

Conclusions: In this group of women with normal glucose tolerance: a) insulin sensitivity changes significantly during the period 24th-28th week of pregnancy since the later oral glucose tolerance is being tested, the higher is insulin resistance (resulting raised of about the 25% from week 24 (18.6 \pm 9.4 SD) to week 28 (14.8 \pm 7.9; $p=0.01$), b) insulin sensitivity appears lower in women who are fatter before pregnancy, independently of gestational weight increase, and finally c) blood pressure is independently related to degree of insulin resistance, similarly to what described in non-pregnant people.

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Lower fasting leptin in gestational diabetes mellitus is related to increased weight gain Al-Daghri N, Bartlett WA, Al-Attas O, Al-Rubean K, Jones AF and Kumar S. Departments of Clinical Biochemistry and Medicine, Birmingham Heartlands Hospital, Birmingham, UK and King Khalid University Hospital Diabetes Centre, Riyadh, Saudi Arabia

Background and Aims: Serum Leptin concentrations are positively correlated with serum insulin concentrations in obese and non-obese subjects. Normal pregnancy is associated with higher leptin concentrations together with hyperinsulinaemia and insulin resistance, but gestational diabetes (GDM) is also associated with impaired insulin secretion. We investigated serum leptin, insulin and weight gain in pregnancy, both in normal women and those with GDM.

Materials and Methods: Thirteen women with GDM, aged [mean (SD)] 32 (5.1) years with a mean body mass index (BMI) 30.8 (5.1) kg/m² and pregnancy duration of 27.7 (3.5) weeks and 59 pregnant women with normal glucose tolerance, aged 30.9 (4.9) years with BMI 30 (6.1) kg/m² ($p=NS$) and pregnancy duration of 26.6 (4.5) weeks were studied. Fasting serum leptin was measured by radioimmunoassay (Linco Research, St. Louis, Mo.) and insulin by ELISA. Leptin concentrations were not normally distributed and were therefore logarithmically transformed: between group comparisons were made by t test.

Results: Leptin concentrations were significantly lower in GDM patients [median (range)] [13.2 (2.7-21.9) ng/mL] than controls [19.1 (3.5-87.0) ng/mL $p<0.0001$]. There was no difference in the baby's weight in GDM [3.85 (0.57) Kg] and control mothers [3.7 (0.46) Kg], but maternal weight gain was significantly higher in GDM mothers [13.1 (3.6) vs 11.1 (3.7) Kg, $p<0.006$]. Multiple regression analysis showed that maternal weight gain ($p<0.02$), insulin concentration ($p<0.018$) and duration of pregnancy ($p=0.04$) were significantly related to the logarithm of the serum leptin concentration ($R^2=0.504$). However, insulin was positively correlated with leptin in normal, but not GDM mothers.

Conclusions: GDM is associated with relative hypoleptinaemia and greater weight gain. This may be related to an altered relationship between insulin and leptin in GDM.

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MEMBRANE LIPID ABNORMALITY IN GESTATIONAL DIABETICS. K. Ghebremeskel¹, C. Lowy², Y. Min¹, B. Thomas¹, B. Offley-Shore², and MA Crawford². ¹Institute of Brain Chemistry and Human Nutrition, University of North London; ²Department of Endocrinology, Diabetes, Guy's, King's and St Thomas' School of Medicine, St Thomas' Hospital, London, SE1 7EH.

Background and Aims: The polyunsaturated fatty acids, arachidonic (AA) and docosahexaenoic (DHA), are vital structural and functional components of cell membranes. In human (type 1 and 2) and experimental diabetes, membrane levels of AA and DHA are depressed. Gestational diabetes mellitus (GDM) is characterised by insulin resistance, impaired glucose tolerance, receptor and vascular dysfunction. But membrane composition is less well characterised. We have investigated membrane lipid integrity in GDM and control women. **Materials and Methods:** Fasting blood samples were obtained from untreated GDM ($n=46$) and healthy controls ($n=44$) after 29 weeks of gestation. Percent fatty acid composition of plasma choline phosphoglycerides (PCPG) and red cell choline (RCPG) and ethanolamine (REPG) phosphoglycerides were analysed by Gas Chromatography and expressed as Mean \pm SD. **Results:** Relative to the controls, the GDMs had higher level of AA (8.5 \pm 2 vs 10.5 \pm 2.2, $P<0.0001$) and comparable DHA values in PCPG fraction. In contrast, in the RCPG of the GDMs both AA and DHA were lower (8.2 \pm 3.0 vs 10.3 \pm 2.1, $P<0.0001$) and (3.3 \pm 1.9 vs 4.9 \pm 1.9, $P<0.0001$) respectively. Similarly, the REPG of the GDMs were lower than the controls (AA, 13.9 \pm 3.4 vs 15.8 \pm 2.1, $P<0.001$) and (DHA, 7.9 \pm 2.3 vs 6.3 \pm 2.8, $P=0.003$). **Conclusions:** Despite adequate levels of AA and DHA in plasma CPG, red cell membrane content of these two fatty acids was reduced in the untreated GDMs compared with women with normal glucose tolerance. These membrane perturbations may account for the recognised alterations in receptor function and impaired transport.

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NORMAL AND GESTATIONAL DIABETES PREGNANCY ARE ASSOCIATED WITH INCREASED PLASMA TNF α LEVELS AND PLATELET AGGREGATION C. Tsigos¹, E. Yfanti¹, I. Elmatzoglou¹, M. Souvatzoglou², T. Demirtzoglou¹, A. Krimizas², I. Kyrou¹, P. Tsiotra¹, E. Papageorgiou³, E. Anastasiou² and S.A. Raptis^{1,3} ¹Hellenic National Diabetes Center, ²Dept. of Endocrinology, Alexandra Hospital and ³2nd Dept. of Internal Medicine, Research Institute and Diabetes Center, University of Athens, Athens, Greece.

Background and Aims: Pregnancy, especially during the second half, is associated with increased insulin resistance, as well as increased coagulability and thrombotic tendency. Increased leptin and TNF α production from the placenta may contribute to both abnormalities. We examined whether platelet aggregation is deranged during pregnancy and whether this might relate to circulating TNF α and leptin levels.

Materials and Methods: We studied 26 women with normal pregnancy (Preg), 30 with gestational diabetes (GDM) and 22 non-pregnant control women (Non-P), all matched for age and BMI. All pregnant women were studied during the 2nd trimester. We measured fasting plasma glucose, insulin, leptin (by RIA) and TNF α (by Elisa), as well as platelet aggregation in response to increasing doses of ADP (1, 2 and 5 μ M/L) in a platelet aggregometer (BIO/DATA Corporation). **Results:** Both normal pregnancy and GDM were characterized by significant increases in platelet aggregation and leptin and TNF α levels compared to the non-pregnant controls (Table), despite their comparable BMIs. TNF α and leptin levels, but not platelet aggregation, correlated significantly with fasting insulin levels ($p<0.05$). Neither TNF α nor leptin levels correlated significantly with platelet aggregation.

	Age	BMI	Leptin	TNF α	ADP1	ADP2	ADP5
	yr	kg/m ²	ng/ml	pg/ml	Aggregation (% increase)		
Non-P	32 \pm 2	27.1 \pm 1.5	11.5 \pm 5	2.3 \pm 0.2	2 \pm 1	10 \pm 2	42 \pm 5
Preg	33 \pm 3	26.5 \pm 1.4	19.8 \pm 3*	3.5 \pm 0.3*	6 \pm 1*	25 \pm 3*	56 \pm 3*
GDM	34 \pm 3	28.0 \pm 1.3	19.0 \pm 4	3.1 \pm 0.2*	9 \pm 2*	34 \pm 4*	64 \pm 2*

* $p<0.05$ vs Non-P; [†] $p<0.05$ vs Preg

Conclusions: Human pregnancy is generally associated with: a) elevations in circulating leptin and TNF α levels, most probably deriving from the placenta and contributing to the development of insulin resistance and b) increased platelet aggregation, which is more pronounced when GDM develops, and apparently contributes to the hypercoagulability observed in these conditions.

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Serum homocysteine is related to glucose tolerance and insulin sensitivity in diabetic and non-diabetic pregnant women.

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Background and Aims: Serum homocysteine levels (sHcy) have previously been found reduced during pregnancy, as well as associated to adverse pregnancy outcomes or early pregnancy losses, while contrasting data exist about sHcy and insulin sensitivity or glucose tolerance in both diabetic and nondiabetic pregnant women. To evaluate this, we measured sHcy in two groups of diabetic and nondiabetic pregnant women.

Materials and Methods: We studied two groups of women classified as nondiabetic (n=35) or affected with gestational diabetes mellitus (GDM, n=25) by means of a 100 g OGTT performed between the 24th and the 28th gestational week, measuring insulin and sHcy both basally and at 2 hours, other than basal serum folate and vitB12.

Results: Both basal and 2-hr sHcy were significantly higher, in the group of diabetic women (5.4 ± 2.25 (SD) $\mu\text{mol/l}$ vs 4.03 ± 1.06 $\mu\text{mol/l}$ and 4.54 ± 2.01 $\mu\text{mol/l}$ vs 3.79 ± 1.07 $\mu\text{mol/l}$; $p < 0.05$ for both). In both groups basal sHcy was higher than 2-hr-sHcy by about the 9% ($p = 0.0001$, by paired t test) and either basal or 2-hr-sHcy were inversely related to serum folate ($r > -0.40$; $p < 0.01$ in both). Only in diabetic women both basal and 2-hr-sHcy were significantly related to 2-hr-plasma glucose ($r = 0.50$; $p = 0.01$ for both), even after adjusting for serum folate ($p = 0.03$). In both groups no relationship was observed between sHcy and serum vitB12, basal or 2-hr-plasma insulin, HOMA indices for insulin sensitivity or beta cell function.

Conclusions: Our findings suggest that: a) GDM is characterised by a significant increase of about the 20% in basal and 2-hr sHcy; b) sHcy appears modified by plasma glucose concentrations, being reduced at 2 hours after a standardised oral glucose load; c) glucose tolerance, as expressed by 2-hr-plasma glucose during the OGTT, is significantly related to both basal and 2-hr sHcy, independently on the prevailing insulin sensitivity only in women with GDM.

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ATTITUDE TOWARDS ORAL GLUCOSE TOLERANCE TEST WITH ONE ALTERED POINT IN GESTATIONAL DIABETES MELLITUS. UTILITY OF A SECOND ORAL GLUCOSE TOLERANCE TEST

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Background and Aims:

According to the criteria of American Diabetes Association (ADA) for the diagnosis of gestational diabetes mellitus (GDM) two or more altered results on oral glucose test tolerance (OGTT) were diagnostic criteria of GDM and those with none altered is normal. However, it is not clear how would to act when there is only one abnormal point. The present study analyses the results obtained in a second OGTT realised in women with one altered result in the first OGTT.

Materials and Methods:

We analysed the results of all OGTT performances to pregnant women during five consecutive years (1996-2000) in our laboratory. According to National Diabetes Data Group, the OGTT is performance with 100 g of glucose administrated oral and in fasting conditions and venous glucose levels are measured at 0, 1, 2 and 3 hours after test. Normal values are 105, 190, 165 and 145 mg/dl respectively. In our hospital we advise to repeat this test when one altered result appears.

Results:

During this five years period we realised 678 OGTT, of whom 178 had one altered result. We performed 88 repetitions of this test and obtained: 30 normal gestant (34.1%), 24 women with one altered result again (27.3%) and 34 women diagnosed as GDM (38.6%).

Conclusions:

The repetitions of OGTT seems necessary because the diagnosis shows an elevated percentage of women with GDM whom could not be diagnosed with the first OGTT and could not be treated adequately. The question, now, is what is the attitude to adopt when in the second repetition one altered result persists.

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IMPACT OF THE CHANGES IN THE GESTATIONAL DIABETES DIAGNOSTIC CRITERIA

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Background and Aims: To assess the impact due to the change of gestational diabetes mellitus (GDM) diagnostic criteria: the currently used National Diabetes Data Group (NDDG) diagnostic criteria in front of the Carpenter and Coustan diagnostic criteria.

Materials and Methods: Data from 1309 pregnant women all of whom were subject to the O'Sullivan test and, when it was indicated, also subject to the oral glucose tolerance test (OGTT), for diagnosis of GDM in our Hospital during the last 5 years (1996-2000) were analyzed.

Results: O'Sullivan: From the 1309 patients subject to the O'Sullivan test, we obtained the following glucose levels, < 130 mg/dL, 131-139 mg/dL and > 140 mg/dL in 609, 151 and 548 patients, respectively. According to NDDG, a 100 g OGTT was performed in 41.9% of these patients. This percentage would then rise to 53.4% when the new Carpenter and Coustan diagnostic criteria are applied.

OGTT: During the same time a total of 678 pregnant women were submitted to the OGTT, resulting that when we applied the NDDG diagnostic criteria, 221 (32.6%) patients are diagnosed as GDM, 178 (26.3%) had only one increased level and 279 (41.1%) were normal. However, when the Carpenter and Coustan diagnostic criteria is applied results show: 316 (46.6%) would have been diagnosed as GDM, 162 (23.9%) would have had only one increased level and 200 (29.5%) would have been normal. With the new diagnostic criteria, there is an increase in the number of women who are diagnosed as GDM. The number of OGTT that we must repeat decrease by 2.4% also decreasing the pregnant women considered normal by 11.6%.

Conclusions: Application of the new GDM diagnostic criteria would involve an increase of the additional OGTT that must be performed. The percentage of patients diagnosed as GDM rises. Therefore, these additional patients must be included in treatment and care programmes which would involve an increase in the global cost and cause inconvenience to the patients. Nevertheless, it must be assessed the clinical repercussion of these changes.

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ISLET AUTOIMMUNITY IS UNCOMMON IN ASIAN WOMEN WITH GESTATIONAL DIABETES.

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Background and Aims: Gestational diabetes (GDM) complicates 3.2% of all pregnancies in Singapore women (ethnic Asian Chinese, Malays and Indians). The associated risk factors for GDM include age, family history and body weight. Affected women may need insulin treatment to achieve normoglycaemia. In most cases, glucose intolerance reverts to normal after childbirth but some are at risk of later development of diabetes mellitus. Islet autoantibodies are causally associated with immune-mediated type 1 diabetes and have also been shown to be detectable in subjects with pre-type 1 diabetes. In this study, we aim to determine the frequency of autoimmunity to islet antigens in our pregnant women with GDM. **Materials and Methods:** We studied a series of 211 consecutive women with historical risk factors for GDM who participated in a glucose tolerance test with 75g oral intake. The 2hr plasma glucose level ≥ 7.8 mmol/l was diagnostic for gestational diabetes. The diabetes-associated autoantibodies tested were ICAs (immunofluorescence), GAD65ab and IA-2ab (radioimmunoassay). **Results:** The group of 211 women comprised of 118 Chinese, 70 Malay and 23 Indian. The glucose tolerance test identified 101 women (47.9%; mean \pm SD age 32.8 ± 5.7 yr) with GDM (59 Chinese, 33 Malay and 9 Indian). Prevalence of seropositivity to either one the 3 autoantibodies were found in 4 (4.0%) women with GDM. One woman was positive for GAD65ab, a 41yr old Chinese with thyrotoxicosis, and required insulin treatment to achieve normoglycaemia. Three women, 2 Chinese and 1 Malay, had detectable IA-2ab and all were on diet treatment. ICAs were not detected in any GDM patient. At six weeks post-natal, 2 of the 4 women with positive autoantibodies remained glucose intolerant. None of the women with normal glucose tolerance had evidence of islet autoimmunity. **Conclusions:** Islet autoimmunity is an uncommon occurrence in Asian women with GDM. A history of GDM is associated with initial presentation or risk of future development of autoimmune type 1 diabetes mellitus in only 4% of the patients.

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FEATURES AND EVOLUTION OF PREGNANCIES COMPLICATED WITH GESTATIONAL DIABETES AND GLUCOSE INTOLERANCE.

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Background and Aims: Gestational diabetes (GDM) is associated with the presence of perinatal complications. The aim is to examine the outcome of the pregnancy in women with GDM and glucose intolerance (GI) compared with pregnant women with normal glucose tolerance. **Materials and Methods:** GDM/GI was diagnosed with 3h 100g-OGTT (24-28, 32-35 weeks). Patients were treated with diet following ADA recommendations, and insulin was added if glucose levels 1 h after meals were >140 mg/dL. **Results:** 4039 pregnancies were attended from 1999 to 2000; 226 women developed GDM/GI (5.6%). GDM/GI patients were older than non-GDM/GI (33.7 ± 4 vs 32 ± 6 years; $p < 0.05$). In GDM/GI patients, family history of diabetes was present in 59.7% and personal history of GDM in 9.3%. Maternal family history was more prevalent than paternal or both together (52.7 vs 36 and 11.3% , respectively; $p < 0.01$). Previous obstetric complications were present in 32%. 54 women received insulin (23.9%). Patients taking insulin developed GDM before patients treated with diet (27 ± 5 vs 31 ± 5 weeks; $p < 0.01$) but pregnancy evolution was the same. HbA1c and fructosamine levels were $4.3 \pm 0.6\%$ and 203 ± 47 mmol/L. Week of delivery was 38.9 ± 1.7 . Delivery was spontaneous in 66% of cases with GDM/GI, and in 90% of cases without GDM/GI ($p < 0.01$). The proportion of cesarean delivery was the same in GDM/GI and non-GDM/GI patients (28% vs 26%). The incidence of fetal macrosomia was higher in GDM/GI group compared with normal group (4.4 vs 1.65% ; $p < 0.05$). Patients submitted to in vitro fertilization (IVF), were more in the group of GDM/GI than in the non-GDM/GI (12 vs 4.8% ; $p < 0.05$). Women with IVF were older (36.4 ± 4 vs 33.3 ± 3 years; $p < 0.05$) and GDM/GI was diagnosed before (28 ± 6 vs 31 ± 5 weeks; $p < 0.05$). Birthweight correlates with mother weight and weight gain during pregnancy ($p < 0.05$). The commonest complication of babies was jaundice (7%). Hypoglycemia was rare (4.4%). In women ($n=49$) with GI the proportion of patients with insulin and pregnancy evolution were the same. We have 26 OGTT after delivery (11.5%): 1 diagnostic of DM and 9 of glucose intolerance. **Conclusions:** Family history of DM (specially maternal) and previous obstetric complications are frequent in GDM/GI women. The proportion of women with insulin and evolution of pregnancies with GDM and GI is similar: good and with a low incidence of complications. The rate of macrosomia is low but higher than observed in newborns of non-GDM/GI women. Birthweight correlates with previous mother weight and weight gain during pregnancy.

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Short term follow-up in Lombardia: preliminary results of the GDM-2000 study
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Background and Aims: The WHO 1999 report on Diabetes Mellitus and the ADA guidelines recommend that women with Gestational Diabetes undergo an OGTT 6-8 weeks after delivery for a correct reclassification of the metabolic state. Several recent reports confirm that compliance of women and of physicians to such recommendations is generally low. A prospective multicentric study in the Region of Lombardia was designed to define the fraction of women with glucose tolerance impairment one year after delivery of pregnancy with GDM. **Materials and Methods:** All women with GDM followed during pregnancy and delivered at each of the 13 participating centres and giving their informed consent were enrolled. Data were recorded during pregnancy on a data sheet containing 169 items including family history for diabetes and personal history of weight, previous and current pregnancy. Mode of treatment and delivery and neonatal data were registered and the women were telephonically contacted to obtain data of post-partum OGTT and one year after delivery OGTT. Over 400 data sheets were collected. **Results:** The short term OGTT at 11.3 ± 7.9 weeks after delivery in 192 women showed that 2% had IFG, 8% had IGT and 4% diabetes. Sixty-two percent were breast feeding at the time of OGTT. Women with abnormal glucose tolerance at follow-up had more frequently first degree relatives with diabetes (58% vs 33%), had more frequently had an abortion (39% vs 20%), had higher pre-pregnancy BMI (27.6 ± 5.8 vs 24.0 ± 4.8), had earlier diagnosis (22.4 ± 7 vs 27.3 ± 5 weeks) with higher plasma glucose at OGTT, had more frequently cesarean delivery (61 vs 21%) had higher capillary glucose levels after delivery (90.1 ± 14.5 vs 80.9 ± 13.1 mg/dl), had heavier babies (3391 ± 472 vs 3198 ± 446 g). **Conclusions:** Glucose tolerance at postpartum evaluation is abnormal in 14% of women with GDM at a mean age of 33 years. Follow-up protocols should be established to better define natural history of GDM and implement measures to possibly prevent disease progression.

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Diabetes and Pregnancy: The Newborn

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NEONATAL HYPOGLYCAEMIA RELATES TO CONTROL IN LABOUR NOT HbA1c IN TYPE 1 DIABETIC PREGNANCY

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Background and aims: The outcome of 109 consecutive pregnancies in type 1 diabetic women managed in a single clinic between 1994 and 1999 were examined to determine predictors of neonatal hypoglycemia and macrosomia. **Methods:** Case record review. **Results:** The mean HbA1c throughout pregnancy was $7.2 \pm 0.8\%$. The duration of diabetes was 12.9 ± 6.8 y and 45 were primigravidae. There was no relationship between neonatal blood glucose (checked before the second feed) and HbA1c at any point in pregnancy or mean pregnancy HbA1c ($R=0.20$; $p>0.1$). However, there was a negative correlation between neonatal blood glucose and maternal blood glucose during labor ($r=-0.25$; $p<0.005$). If maternal blood glucose during labor was greater than 9mM, neonatal blood glucose was always less than 2.5 mM (mean 1.7 ± 0.4 mM). There was no relationship between mean HbA1c and birthweight ($R=0.02$; $p>0.1$) nor maximum insulin dose and birthweight ($R=0.09$; $p>0.1$). Fetal abdominal circumference measured by ultrasound at 34 weeks correlated strongly with birthweight ($R=0.72$; $p<0.001$) and abdominal circumference over 340mm was a strong predictor of macrosomia (mean birthweight 4229 ± 157 g). **Conclusions:** Neonatal hypoglycemia correlates with maternal hyperglycemia in labor, not with HbA1c during pregnancy in the range of control achieved. Macrosomia does not correlate with HbA1c during pregnancy. Fetal abdominal circumference at 34 weeks predicts macrosomia and complications at delivery.

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Diabetic pregnancy and fetal congenital malformations

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Maternal diabetes induces changes in the metabolism of the fetus and in turn causes a number of complications resulting in malformations. The frequency of anomalies in fetuses of diabetic pregnant women ranges from 4-9%, in comparison with the overall population from 1-2%. **The aim of our study** is an analysis of the prevalence and possible causes of fetal malformations in diabetic pregnancies. To determine it, we followed two groups of newborns delivered in our department by patients with pregestational diabetes over the two periods: first-1988-93 (N=209) and second-1994-1999 (N=198) and 4700 infants born to healthy mothers in this second period in our hospital (control group). Among newborns delivered in the first of analyzed periods 13 (6.2%) manifested congenital malformations, but in the second group 17 (8.58%) (p-NS). In the control group the number of malformations was significantly lower- 3.8% (odds ratio 2.26; $p<0.05$). Glycated hemoglobin (HbA1c) level during the first trimester was not significantly higher in women whose infants were malformed. We proved however, that when the HbA1c value exceeded considerably the standard ($>9.3\%$) the risk of malformations was greater. There was however a strong correlation between malformations rate (MR) and mean glucose levels in the first trimester of pregnancy. The malformations rates were significantly higher among offspring of mothers with mean glycemia above 100 mg/dl, than among those women in whom this level remains below 100 mg/dl (odds ratio 1.9; $p<0.05$). The MR in White Classes D-II was higher, but not significantly than in classes B-C. The most frequent anomalies were as follows: cardiovascular, genitourinary, central nervous system and skeletal. **Conclusion:** Our results confirm that diabetic pregnancy despite the better metabolic control still induces alterations in fetal development, but especially in the group of patients with tendencies to very hesitant glucose levels.

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EFFECT OF SOCIAL STRESS AND GESTATIONAL INSULIN DEFICIENCY ON GLUCOSE HOMEOSTASIS IN MALE OFFSPRING
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Background and Aims: We have previously shown that maternal social stress (MSS) enhanced gestational insulin deficiency (GID)-induced glucose intolerance in Wistar rats male offspring (F₁) at puberty. The study was conducted to evaluate the impact of stress and gestational insulin deficiency on glucose homeostasis in male F₁ at the sex-maturity period. **Materials and Methods:** For MSS creation rats were transferred daily from one association to another within 2nd-8th day of pregnancy, GID was rendered by a single streptozotocin injection (45 mg/kg b.w., i.p.) on the second day of pregnancy. The maternal cohort consisted of 50 pregnant rats exposed to MSS, GID, MSS+GID and controls (C). An i.p. GTT (3g/kg b.w.) was performed in male F₁ (n=32) at 90 days of age. Liver glycogen (GI) contents, glucose-6-phosphatase (G-6-Ph) activity and fasting plasma NEFA levels were measured in F₁ by spectrophotometry. **Results:** Decrease in GI contents observed in GID F₁ was more pronounced in MSS+GID F₁: 26.0±1.7 vs 15.6±1.1 mg glucose/kg, respectively (p<0.001), in comparison with 45.7±2.0 mg glucose/kg in CF₁ (p<0.001). Plasma glucose (AUC/2h over GTT), NEFA levels and liver G-6-Ph activity in the MSS+GID F₁ were increased by 10.4, 22.4 and 22.2 % respectively (p<0.01), compared to the GID F₁ and by 141.3, 89.2 and 102.6 % respectively (p<0.001), compared to the CF₁. **Conclusions:** Thus, maternal social stress strengthened glucose homeostasis disturbances induced gestational insulin deficiency in first generation rats male offspring at the sex-maturity period.

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INSULIN RESISTANCE DEVELOPMENT IN OFFSPRING OF STRESSED GESTATIONAL DIABETIC RATS

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Background and Aims: It was revealed insulin resistance in adult offspring (F₁) of pregnant streptozotocin diabetic rats. The effect of gestational diabetic mothers stressing on insulin sensitivity in F₁ is still unknown. The aim of the study was to investigate the impact of maternal social stress (MSS) against the background of gestational diabetes (GD) on insulin resistance and beta-cell function in rats male F₁ at puberty. **Materials and Methods:** For MSS creation rats were transferred daily from one association to another one within 2nd-8th day of pregnancy, GD was rendered by streptozotocin injection (45 mg/kg b.w., i.p.) on the 2nd day of pregnancy. The maternal cohort (n=25) was divided into 4 groups exposed to MSS, GD, MSS+GD and controls (C). An i.p.GTT (3 g/kg b.w.) was performed in F₁ (n=32) at 45 days of age. Fasting plasma samples were analyzed for NEFA by colorimetry and insulin by RIA. Homeostasis Model Assessment (HOMA) was used to estimate beta-cell function (BCF) and insulin resistance (IR). **Results:** Plasma glucose, IRI and NEFA levels in MSS+GD F₁ were increased in comparison with GD F₁ (AUC/2h over GTT: 1118.4±15 vs 933±21 mmol·l⁻¹; IRI: 300.0±4.3 vs 230.3±4.3 pmol/l; NEFA: 820±51 vs 650±47 μmol/l, respectively, p<0.05) and CF₁ (AUC/2h over GTT: 716±11 mmol·l⁻¹; IRI: 71.4±4.0 pmol/l; NEFA: 370±61 μmol/l, respectively, p<0.001). HOMA-IR and HOMA-BCF indices were enhanced in both GD F₁ and MSS+GD F₁ compared to CF₁, but more pronounced in MSS+GD F₁ (IR: 7.9±0.3 and 9.7±0.39 vs 2.4±0.09 in CF₁, respectively, p<0.001; BCF: 511.4±46.7 and 914.2±168.8 vs 392.3±73.9 in CF₁, respectively, p<0.05). **Conclusions:** The maternal social stress heightened GD-induced insulin resistance and strengthened beta-cell function in the first generation rats male offspring at puberty.

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Without confounding factors, birthweight remains a strong determinant of metabolism even in prematurity

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Background and Aims: The relationship between birthweight and disease in later life is well-established, although studies which repudiate this relationship still appear. In South Africa not only is low birthweight common, but hypertension, obesity and diabetes are on the increase. In addition premature birth is also common. This study aimed to establish the relationship of the thrifty phenotype with prematurity, but also to start unravelling considerations for the design of interventions.

Materials and Methods: We studied 85 infants (46 girls and 39 boys) between the 1st and 60th day of life (median 17 days). Each individual was given a standardised milk feed (21ml/kg Nan®) after a 4 hour fast. Blood was taken using a heelprick at fasting and 60 minutes after the feed. Each child was measured at birth and at the time of the tolerance test: parameters were weight, length, head circumference, triceps and subscapular skinfold thickness, and crown-rump and sacrum-heel lengths. The ponderal index was calculated at both study times.

Results: Birthweight and weight at time of the meal tolerance correlated negatively with the glucose at 60 minutes (r=-0.34, p=0.002 and r=-0.33, p=0.005). Weight at the time of the tolerance test correlated with the fasting insulin (r=-0.35, p=0.001). Sacrum-heel length at birth correlated negatively with 60 minute glucose (r=-0.37, p=0.01). Birthweight was the strongest determinant of the glucose concentration. Weight velocity correlated negatively with birthweight (r=-0.55, p<0.005) and HOMA (r=-0.32, p<0.01), independent of one another. The lowest birthweight children had the highest weight velocity and/or the greatest insulin sensitivity (lowest weight velocity quartile vs highest quartile: 0.16kg/day vs 0.007 kg/day p<0.0001. HOMA, lowest quartile vs highest: 0.23 vs 0.94, p<0.0001). A proxy measure of "brain sparing", head circumference: birthweight, correlated with insulin sensitivity (r=0.76, p<0.05).

Conclusions: These data show a clear relationship between birthweight and glucose tolerance. In addition insulin sensitivity is a strong determinant of the neonatal response to low birthweight. Whilst this suggests that intervention at this stage is possible, one must take the other possible consequences into consideration. For example, in a separate study of children aged seven years, we showed that growth velocity beyond that predicted by the birth percentile had a detrimental effect upon glucose tolerance. Thus intervention needs to be planned with care lest the treatment outweighs the effect of low birthweight.

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PREDICTORS FOR NEONATAL HYPOGLYCEMIA IN LARGE-FOR-GESTATIONAL-AGE NEWBORNS

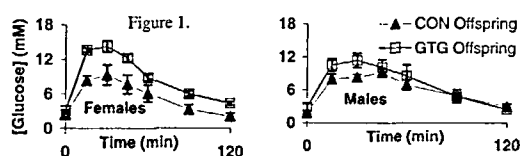
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Background and Aim: Testing for neonatal hypoglycemia is recommended for large-for-gestational-age newborns (LGA). We investigated the rate and timing of hypoglycemia in LGA newborns and whether any maternal or neonatal parameters were predictive. **Materials and Methods:** In a retrospective study of 1094 LGA newborns (birth weight ≥ 90th percentile) maternal parameters (age, parity or BMI; history of macrosomia, stillbirth or gestational diabetes (GDM); current GDM (n=148), C-section and gestational age (GA) at delivery) and neonatal parameters (Apgar/5 min, cord pH, ratio of head/abdominal circumference, rate of neonatal BMI or Ponderal Index ≥ 90th or LGA ≥ 95th percentile) were correlated with the rate of neonatal hypoglycemia, defined as capillary glucose ≤ 30mg/dl. Glucose testing was performed at 1 hour of life (H) with subsequent evaluations based on clinical findings. Deliveries before 34 weeks of GA were excluded. Stepwise regression and ROC analysis was used to evaluate predictors. **Results:** Hypoglycemia within the first 24 hrs occurred in 16.3% (178), intravenous (IV) glucose was required in 12 % of these (21/178). 70.8% of the cases were diagnosed at the 1H, 7.8% at 2H, 2.8% at 3H, 3.9 % at 4H and 14.6 % in the remaining 20 H. IV-glucose was required less frequently with 1H hypoglycemia versus later (8.7 vs 19.2%, p= 0.04). GA at delivery was the only predictor (p= 0.002) with the threshold for prediction at 39 weeks (48.7 vs 33.8% for <39 compared to ≥39, p<0.0001). In a subgroup analysis of 306 women with antenatal oGTT performed the 1 hr post challenge glucose value was the only predictor (p< 0.0001). The best cut offs were 120 mg% (0% vs 15.9% for < 120 compared to ≥ 120, p= 0.005) and 200 mg% (10.0 % vs 26.8% for < 200 compared to ≥ 200, p= 0.0004). **Conclusion:** Routinely postnatal testing of LGA newborns appears to be indicated with a 16% prevalence of neonatal hypoglycemia. No clinically useful maternal or neonatal predictors for hypoglycemia were identifiable unless antenatal oGTT testing was performed. The 1H oGTT value was useful to discriminate between newborns at minimal, intermediate and high risk for hypoglycemia.

GLUCOSE INTOLERANCE IN OFFSPRING OF DIABETIC MICE

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Background and Aims: Fetal environment and genetic influences may predict health in adult life. The aim of this study was to investigate the effects of maternal hyperglycaemia and hyperinsulinaemia on the glucose tolerance of offspring independent of their genetic susceptibility to diabetes. **Materials and Methods:** 56 CBA/T6 mice (28 females) were injected with either goldthioglucose (GTG) (0.5mg/g body weight) or saline (CON). 6 weeks post-injection, mice were subjected to an oral glucose tolerance test (OGTT, 50% glucose, 3g/kg). 4 breeding groups (each of 7 pairs of the 4 possible combinations) were set up 1 week later. At 12 weeks of age the offspring of these matings were weighed and subjected to an OGTT. **Results:** No offspring resulted from any breeding pairs that included GTG males. The CON breeding pairs produced 3 litters (9 female, 7 male) and the GTG female/CON male breeding pairs produced 1 litter (7 female, 2 male). Prior to breeding, every GTG female was significantly heavier and more glucose intolerant than the CON females (>2 SD's above mean). 12 week old female offspring of GTG mothers had significantly higher body weights than the female offspring of CON mothers ($19.5g \pm 0.4$ cf $18.0g \pm 0.4$, $p = 0.01$). The body weights of the male offspring were similar ($22.5g \pm 2.6$ cf $22.3g \pm 0.5$, $p = 0.94$). The glucose tolerance of the female offspring from GTG mothers was impaired compared to those from the CON mothers (Total AUC = $1070\text{mM}\cdot\text{min} \pm 52$ cf $662\text{mM}\cdot\text{min} \pm 112$, $p = 0.01$, Figure 1), and no difference was seen in the male offspring (Total AUC = $875\text{mM}\cdot\text{min} \pm 70$ cf $741\text{mM}\cdot\text{min} \pm 65$, $p = 0.25$). **Conclusions:** Independent of genetic diabetic susceptibility, fetal environment did predispose the female offspring of overweight, diabetic mice to gain more weight and develop glucose intolerance.



Current Weight, not Birth Weight of 'Catch-up' Weight, Accounts for Insulin Resistance in Healthy Five-Year-Olds
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Background and Aims: The 'Fetal Origins' hypothesis is predicated on well-established associations between low birthweight (BW), insulin resistance (IR) and metabolic disturbances later in life. Gestational quality, however, has greatly improved since the original Barker studies. Furthermore, BW correlates as much with post-natal as with pre-natal size, so that W achieved at 5 years may be as important a risk factor for IR as poor gestation. The objective is to establish the relative contributions of current W, W 'catch-up' (centile-crossing) and BW to metabolic status at 5 years of age.

Materials and Methods: EarlyBird is a prospective cohort study of 300 healthy school entrants. Data on baseline anthropometry and IR by homeostasis model assessment (HOMA) are presented here from the first 100 recruits (mean age 4.8 years), and BWs from 2017 contemporary births of 1995 to quantify low BW frequency.

Results: Only 2.5% of the 217 births were of low BW ($<2500g$) at term. There was no correlation between IR at 5y and BW in the study cohort ($r=0.07$). IR & current W correlated ($r=0.41$, $p<0.001$) as did IR & W 'catch-up' (number of W SDS crossed) at age 5y ($r=0.31$, $p<0.05$), though only in girls. However, W 'catch-up' did not improve on (ie co-correlated with) current W in the prediction of IR. Most importantly, IR was the same in children of lighter BW who experienced 'catch-up' as in those of heavier BW matched for current W at 5y, whose W SDS had not changed. IR, even at 5y but again only in girls, correlated with glucose and LDL cholesterol.

Conclusions: 1) Low BW at term is now rare in the UK and probably irrelevant to IR (Barker's studies were based on low BW frequencies reaching 10% in the pre-war UK cohorts). 2) IR in the contemporary 5-year-old is unrelated to BW, and is best predicted by current W. 3) Centile-crossing appears to be more a phenomenon of excess weight-gain than one of physiological 'catch-up'. 4) 'Catch-up' W does not add to current W in the prediction of IR. 5) The implication that the IR-related metabolic disturbances already present at 5y are (avoidably) related to the early overfeeding of healthy infants will be of importance to public health policy-makers in modifying nutritional behaviours.

Correlation between low birth weight and obesity/overweight in the adult age.

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Background and Aims: Clinical correlates of metabolic syndrome (MS) such as obesity, impaired glucose tolerance, hypertension or dyslipidaemia are singularly or globally related to a small birth weight in agreement with the hypothesis of 'thrifty phenotype'. Since there are, however, conflicting data about the entity of such relationship, this study was conceived to evaluate in our population the grade of association of birth weight with entity of overweight and co-presence of one or more correlates of MS in adult age.

Materials and Methods: We measured fasting plasma glucose, triglycerides and blood pressure in 357 overweight-obese outpatients (163M/194F) with BMI >25 kg/m² who consecutively came to our observation and whose birth weight and at age of 18 years were precisely known, being this group compared with 42 normal-weight age and sex matched controls. MS was scored as 0: (MS-0) if at the time plasma glucose was <6 mmol/l, systolic blood pressure was <140 mmHg or was diastolic <90 mmHg, and triglycerides were <1.92 mmol/l; scored as 1: (MS-1) or 2: (MS-2) if one or respectively two of more above mentioned aspects were present.

Results: No difference in mean birth weight value was observed in the overweight group (BMI between 25 and 30 kg/m², $n=178$, 3682 ± 740 (SD) gr) as compared to the obese group (BMI >30 kg/m², $n=179$, 3656 ± 816 gr) or to normal-weight controls ($n=42$; 3602 ± 642 gr; p ns after ANOVA). Birth weight was significantly lower in MS-2 patients ($n=6$; 2883 ± 1686 gr) as compared to MS-0 ($n=208$, 3670 ± 774 gr) and MS-1 patients ($n=143$; 3712 ± 879 gr; $p<0.05$, after ANOVA); being the odds ratio of having a full-blown MS in those with a birth weight <2500 gr = 7.8 (CI95% 2.25-27.04) after adjusting for age and sex ($p=0.01$). Finally birth weight was unrelated to BMI, blood pressure, and plasma lipids, resulting weakly related with BMI at 18 years ($r=0.13$; $p>0.05$).

Conclusions: according to the present results a) obesity seems to be 'per se' unrelated to a small birth weight, b) obesity or overweight are associated with a small birth weight only in presence of at least two correlates of MS (impaired glucose tolerance, hypertension or dyslipidaemia).

THE PHENOTYPE EXPRESSION IN WOMEN WITH DIABETES MELLITUS WITH OR WITHOUT FETAL MACROSOMIA

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Background and Aims: Fetal macrosomia is a well-known risk factor for diabetes. The aim of this study was to establish if there are any phenotypic differences between diabetic women who gave birth to macrosom or normosom children.

Materials and Methods: The study cohort comprised 560 diabetic women divided into two groups: Group I: 279 women with macrosom births (birth weight >4000 g) - 35 T1DM/244 T2DM, mean age 58.1 ± 10.8 yr. and Group II: 281 women with normosom births - 40 T1DM/241 T2DM, mean age 58.9 ± 11.5 yr. We made a retrospective analysis of the clinical and metabolic characteristics of the two groups. For statistical analysis we used χ^2 and Student's t tests.

Results: We found significant differences between the two groups for the following parameters: age at diabetes onset (48.4 ± 13.2 yr. for Group I, 51.4 ± 12.3 yr. for Group II, $p=0.01$); maternal history of diabetes (%) (57.5 ± 24.4 vs. 76.8 ± 17.8 , $p=0.0001$); prevalence of oral agent treatment (%) (48.7 ± 25 vs. 6.4 ± 6 , $p=0.0001$); BMI (29.7 ± 5.3 vs. 28.7 ± 4.7 , $p=0.05$); waist circumference (cm) (104 ± 13.5 vs. 97.4 ± 9.2 , $p=0.0001$); HbA1c ($11 \pm 2.4\%$ vs. $10.3 \pm 2.6\%$, $p=0.002$); total cholesterol (mg/dl) (229.6 ± 37.6 vs. 215.6 ± 72.1 , $p=0.0001$); HDL Chol. (59.5 ± 6.3 vs. 37.6 ± 7 , $p=0.0001$); LDL Chol. (139.5 ± 27.7 vs. 149.6 ± 46.5 , $p=0.01$); triglycerides (mg/dl) (153.2 ± 17.8 vs. 141.3 ± 90.4 , $p=0.05$); albumin excretion rate (mg/24h) (63.7 ± 61.2 vs. 25.7 ± 16.4 , $p=0.0001$); diabetic nephropathy prevalence (%) (13.75 ± 11.86 vs. 6.4 ± 6 , $p=0.01$); prevalence of arteriopathy (%) (25 ± 18.7 vs. 6.4 ± 6 , $p=0.0001$).

Conclusions: The phenotype characteristics of women with diabetes who gave birth to macrosom children are: a lower age at onset of the diabetes and the presence of the main features of insulin resistance syndrome suggesting that this is the main underlying pathologic mechanism of fetal macrosomy in diabetic women.

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A Survey on Contraception in a Cohort of Italian Diabetic Women. Napoli*, A.Colatrella*, R.Botta*, G. Di Cianni, F.Fallucca*, D. Fedele*, R.Fresa*, S.Gamba*, S.Italia*, D.Mannino*, I.Piva □, C.Suraci*, L.Tonutti*, E.Torlone*, C.Tortul*, A. Lapolla*.
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In the space of one year (from 1999 to 2000), a survey on contraception and obstetrical history was performed on a cohort of fertile diabetic women.

Methods: we distributed an anonymous questionnaire to 667 Caucasian diabetic women (466 IDDM and 201 NIDDM, $c^2=0.0001$) attending 13 diabetes centres located throughout the national territory. Patients' characteristics: age 34.75 ± 8.9 yr (range 17-52 yr) (IDDM 32.09 ± 7.6 vs NIDDM 42.01 ± 8.5 , ANOVA $p=0.0001$), BMI 24.9 ± 5.1 (IDDM 23.19 ± 3.7 vs NIDDM 29.3 ± 5.7 , ANOVA $p=0.0001$), diabetes duration 12.41 ± 8.85 yr (IDDM 14.1 ± 9 vs NIDDM 7.5 ± 5.8 , ANOVA $p=0.0001$). Each centre was asked to contribute to the study, with at least 50 questionnaires; as a result 37.03% of surveys were gathered in the north, 36.43% in the centre and 26.43% in the South of Italy.

Results 96.85% of our population had their first sexual intercourse at the age of 19.68 ± 3.16 . Contraception: 30.35% of these women used hormonal contraceptives, 11.99% IUD, 9.59% declared they used no contraception, 48.2% only utilised barrier and/or natural methods. However, irrespective of their previous contraceptive strategy (pill, IUD, barrier, natural), 8.84% of all the studied population was surgically sterilized during a caesarean section. Pattern of contraceptive practice: 54.2% of the women chose their contraceptive practice autonomously and/or with their partners, 33.9% received their advice from gynaecologist, 4.4% from diabetologist, 7.1% from both the specialists, 0.4% from G.P. or others. Among those who chose a hormonal contraception, 60.4% were prescribed by a gynaecologist, 11.2% by a diabetologist, 15% by both of them, 13.4% by others. Diabetes treatment: 29.95% of insulin treated women and 27.61% of women treated with diet and/or oral hypoglycaemic agents used O.C. Smoking habits: 26.9% of women taking hormonal contraception were smokers. Educational level: 36.62% (26/71) graduated, 32.42% (95/293) high school, 28.37% (59/208) secondary school, 15.5% (7/45) primary school used O.C. Obstetrical History: The average of deliveries was 1.14 ± 1.1 (I.C. 195% = 1.1-1.2), of miscarriages was 1.3 ± 0.7 (I.C. 195% = 1.2-1.4) and of induced abortions was 1.24 ± 0.5 (I.C. 195% = 1.1-1.2). Planning of at least one pregnancy, was reported in 29.8% patients.

Conclusion: future work needs to increase the role of diabetologist in educating fertile diabetic women about contraception and pre-pregnancy counselling

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THE EFFECT OF BODY MASS INDEX ON HbA1c LEVELS IN NORMAL AND GESTATIONAL DIABETIC PREGNANT WOMEN

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Background and Aims: There are no sufficient data regarding the effect of the BMI of women before pregnancy on the HbA1c levels during normal and gestational diabetic (GDM) pregnancy. This study assesses HbA1c levels according to prepregnancy BMI and trimester (tr) in normal and GDM pregnancy. **Materials and Methods:** In accordance with ADA-2000 criteria, 2249 were Normal (N) (1st tr n=105, 2nd tr n=1140, 3rd tr n=1004) and 1079 were diagnosed as GDM. From the GDM group 656 women were treated with diet only (GDM-D) (2nd tr n=283, 3rd tr n=373) and the remaining were treated with insulin as well (GDM-I) (2nd tr n=235, 3rd tr n=188). All pregnant women were divided in three subgroups according to prepregnancy weight: (i) Normal weight group (BMI1) $18.5-24.9$ kg/m², n=1829; (ii) Overweight (BMI2) $25.0-29.9$, n=884; (iii) Obese (BMI3) >30 , n=515. In all pregnant women HbA1c levels were measured (HPLC-Merian) at the time the OGTT was performed. **Results:** The HbA1c (%) levels per diagnosis, trimester and BMI subgroup were: N: 1st tr BMI1: 4.1 ± 0.4 , BMI2: 4.1 ± 0.4 , BMI3: 4.2 ± 0.3 , 2nd tr BMI1: 3.8 ± 0.4 , BMI2: 4.0 ± 0.4 , BMI3: 4.1 ± 0.4 , 3rd tr BMI1: 4.0 ± 0.4 , BMI2: 4.1 ± 0.4 , BMI3: 4.1 ± 0.4 , GDM-D: 2nd tr BMI1: 3.9 ± 0.4 , BMI2: 4.1 ± 0.4 , BMI3: 4.2 ± 0.4 , 3rd tr BMI1: 4.2 ± 0.5 , BMI2: 4.3 ± 0.4 , BMI3: 4.5 ± 0.5 , GDM-I: 2nd tr BMI1: 4.4 ± 0.7 , BMI2: 4.4 ± 0.6 , BMI3: 4.6 ± 0.6 , 3rd tr BMI1: 4.6 ± 0.7 , BMI2: 4.9 ± 0.8 , BMI3: 4.9 ± 0.6 . HbA1c levels were significantly higher ($p<0.001$) GDM-I compared with N and GDM-D as well as GDM-D compared with N ($p<0.05$) in all respective BMI subgroups in the 2nd and 3rd tr. In N, GDM-D, GDM-I groups HbA1c levels were significantly higher ($p<0.001$) in the 3rd tr compared to the 2nd in all BMI subgroups. Finally, in all categories HbA1c levels were significantly higher in the BMI3 subgroup in the 2nd tr and 3rd tr ($p<0.001$) compared to BMI1. **Conclusions:** At the time of diagnosis GDM women, and especially the insulin treated group, had significantly increased HbA1c levels. Obese pregnant women irrespectively of glucose tolerance had significantly higher HbA1c levels. Hence, the optimal HbA1c targets for women with pre-gestational and gestational DM have to be defined according to prepregnancy BMI and trimester of pregnancy.

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ELEVATED HAEMOGLOBIN A1C IN THE THIRD TRIMESTER IS RELATED TO PRETERM DELIVERY IN TYPE 1 DIABETIC WOMEN.

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Background and Aims: High HbA1c in early pregnancy and elevated urinary albumin excretion (UAE) ≥ 30 mg/24 h have been associated with preterm delivery in type 1 diabetes. However, the influence of metabolic control during pregnancy on the increased risk of preterm delivery is not well described. The aim was to investigate the importance of HbA1c at 28 weeks of gestation in relation to HbA1c in early pregnancy as a predictor of preterm delivery in women with type 1 diabetes. **Materials and Methods:** A prospective study including 213 consecutive pregnant women with type 1 diabetes and normal UAE. Miscarriages (<22 weeks of gestation), twin deliveries and pregnancies beyond first delivery in the inclusion period were excluded. HbA1c (normal range 4.1-6.4%) at 10 and 28 weeks of gestation were used for analysis. Pre-eclampsia was defined as development of BP $>140/90$ accompanied by proteinuria >300 mg/24h later than 20 weeks. Preterm delivery was defined as delivery before 37 completed weeks. **Results:** Seventy women (33%) delivered preterm and 143 at term. The 2 groups were comparable regarding age, BMI, duration of diabetes, retinopathy, BP, and parity. HbA1c at 10 weeks was $7.3(1.0)$ vs. $6.9(0.9)\%$ ($p<0.01$) and at 28 weeks $6.7(0.8)$ vs. $6.1(0.7)\%$ ($p<0.001$) and development of pre-eclampsia occurred in 11% vs. 3% ($p<0.05$). Using multivariate logistic regression analysis (after testing for linearity) HbA1c at 28 weeks was the only independent variable associated with preterm delivery ($p>0.001$). The odds ratio pr. 1% increment in HbA1c at 28 weeks was 3.1 (CI 2.0-4.9). **Conclusions:** HbA1c at 28 weeks is the best predictor of preterm delivery in women with type 1 diabetes and normal UAE.

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LONGER GESTATION PERIOD AND BETTER GLYCEMIC CONTROL WITH INSULIN LISPRO TREATED PREGNANCIES IN TYPE 1 DIABETES

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Background and Aims: To determine the safety and efficacy of insulin lispro during pregnancy.

Materials and Methods: All women with type 1 diabetes were followed during their pregnancy at the Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center. Care was delivered by a multidisciplinary team that included endocrinologists, nurse educators, nutritionists, and social workers. Subjects received either insulin lispro (LP) or regular human insulin (R) as a short acting insulin. Ultra Lente or NPH were the basal insulin received. Ninety-five pregnancies (33 using R and 62 using LP) were closely followed for diabetes care parameters, progression of diabetic retinopathy and albumin excretion rates (AER), development of toxemia of pregnancy and fetal outcome (especially for birth weight, length of gestation and the need for caesarian sections).

Results: Mean age and duration of diabetes in the two treatment groups were similar. Mean glycosylated hemoglobin values were significantly lower throughout pregnancy in the insulin lispro treated subjects when compared with the R treated group (7.0% vs. 8.6% around conception, 6.0% vs. 7.3% at 20 weeks, and 5.9% vs. 7.3% at the end of the pregnancy). Fetal birth weight when controlled for gestational age was not significantly different in the LP group when compared with the R treated group. However, the LP treated pregnancies had a significantly longer gestational period (36.9 ± 0.2 vs. 35.6 ± 0.5 wks, $p<0.04$, t-test). The number of severe hypoglycemic episodes was significantly lower during the LP treated pregnancies (0.56 ± 0.16 vs. 1.55 ± 0.38 episodes/patient, $p=0.02$, t-test). Mean eye grades before and after delivery were better for the LP treated group.

Conclusion: We conclude that better glycemic control can be achieved with significantly less hypoglycemia, and the gestational age is significantly longer in insulin lispro treated pregnancies.

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Abnormal glucose challenge test predicts fetal macrosomia in normotolerant pregnant women

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Background and Aims: In infant of women with Gestational Diabetes Mellitus (GDM), fetal overgrowth is thought occur because of fetal hyperinsulinemia due to an excess supply of nutrients such as glucose. Whether the fetal macrosomia is present also in infants of mothers with abnormal oral glucose challenge test (GCT) in absence of GDM is not clear. Aim of this study is to evaluate maternal-neonatal morbidity in pregnant women with an abnormal 50-g 1-hr Glucose Challenge Test (GCT) and a normal 100-g, 3-hr OGTT, who are not considered to have GDM.

Materials and Methods: This study retrospectively evaluates the neonatal and maternal outcome in normotolerant pregnant with abnormal GCT (GCT+). The results were compared with a group of 491 women (CON), with normal GCT, matched for age, parity, family history of diabetes, prepregnancy body weight and BMI, pregnancy weight gain and gestational week at screening test.

Results: Screening for Gestational Diabetes with a Glucose Challenge Test (GCT-50 g) was performed in 3159 women: 1089 (36.4%) had abnormal GCT (1 hr plasma glucose ≥ 140 mg/dl) and 622 (19.6%) had normal glucose tolerance after three-hour glucose tolerance test. The relationship between GCT and birth weight was then assessed in 365 women out of the latter 622 women (GCT+). Their main clinical features were: 32 \pm 4.6 years old, primiparous 52.3%, prepregnancy weight 60.1 \pm 5.7 kg, BMI 22.5 \pm 3.8 Kg/m², weight gain 8.1 \pm 5.4 Kg. GCT+ showed an higher incidence of macrosomia (10.2% vs 9.1%; $p < 0.0001$) and Large for Gestational Age (LGA: 18.4% vs 14.8; $p < 0.0001$). The incidence of Caesarean Sections as well was higher in GCT+ (25.7% vs 24.1%; $p < 0.0001$). In GCT+, birth weight correlated with prepregnancy weight ($r = 0.22$; $p < 0.0001$), maternal weight gain ($r = 0.15$; $p < 0.001$) and plasma glucose values observed at fasting ($r = 0.13$; $p < 0.01$), after GCT ($r = 0.14$; $p < 0.03$) and GCT increment above baseline ($r = 0.16$; $p < 0.03$). On multivariate analysis only prepregnancy weight and maternal weight gain remained significantly associated with birth weight (r^2 0.77). **Conclusion:** we confirm that caucasian women with an abnormal GCT but normal 100-g OGTT, i.e. non diagnostic Gestational Diabetes, have an increased incidence of macrosomia. Prepregnancy weight and weight gain are determinant of the risk of macrosomia in women with non diagnostic test for Gestational Diabetes

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Characteristic features of the metabolic syndrome in women with prior GDM: Circulating leptin concentrations during oGTT.

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Background and Aims: Gestational diabetes (GDM) is known to be a predictor or an early manifestation of the metabolic insulin resistance syndrome. Our aim was to study the clinical and laboratory constituents of this syndrome and their relationship with circulating leptin during an oGTT in women with prior GDM.

Materials and Methods: BMI, waist-to-hip ratio (W/H), blood pressure, PAI 1, fibrinogen, HbA1c, lipoprotein lipids (total-, HDL- and LDL-cholesterol, triglycerides) and uric acid were measured in 54 women with prior GDM at a follow-up investigation 3.4 \pm 0.4 [\pm SD] yrs after delivery. Their mean age was: 35.8 \pm 5.7 yrs; BMI: 27.8 \pm 7.1 kg/m²; 25 of them were on insulin during index pregnancy, and 25 had GI (DM or IGT or IFG) at reclassification (WHO criteria). Blood glucose (BG), immunoreactive insulin (IRI; RIA), C-peptide (CP; RIA) and leptin (RIA, normal values in women: < 11.1 ng/l) concentrations in the fasting state and during a 75 g oGTT were also determined. A metabolic score (1 to 3; higher values at the pathological end) using tertiles of W/H, systolic blood pressure, PAI 1, fibrinogen, HbA1c, uric acid, HDL-cholesterol and fasting IRI was calculated. For statistical analysis different correlation methods and multiple logistic regression were used.

Results: An elevated fasting leptin level (18.9 \pm 12 vs. 15.0 \pm 9.5), and a slight, but non-significant increase of the 120min leptin level (22.7 \pm 15.4 vs. 16.9 \pm 9.6) in the GI group compared to metabolically healthy women was observed during the 180min oGTT. The analysed fasting, 90min and 120min leptin were significantly higher in patients with a metabolic score above 2 ($n = 24$; $P < 0.005$). These leptin values were also associated with BMI ($P < 0.001$), W/H ($P < 0.01$), HDL-cholesterol ($P < 0.05$), HbA1c ($P < 0.05$), fasting and 120min IRI ($P < 0.05$), and gamma-glutamyl transferase values ($P < 0.01$). Using multivariate analysis the independent covariates of the different leptin levels were: W/H and PAI 1 for fasting leptin, HDL-cholesterol and 120min IRI for 90min leptin, and W/H and 120min IRI for 120min leptin. Other variables available for the model were: fibrinogen, fasting IRI, and HbA1c.

Conclusions: The associations found suggest that circulating leptin may have a role in the metabolic syndrome in women with prior GDM. 120min IRI might be a factor or a marker in the development of the insulin resistance syndrome, determining also changes in circulating leptin during oGTT.

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THE EFFECT OF MICRO- AND MACROALBUMINURIA ON THE COURSE OF PREGNANCY AND PERINATAL OUTCOME.

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Background and Aims: To study the effect of micro- and macroalbuminuria on the course of pregnancy and perinatal outcome. **Materials and Methods:** Sixty-six women with pregestational diabetes mellitus (Type 1) were enrolled to the study. Strict metabolic control and fetal surveillance were performed. Pre-pregnancy microalbuminuria was detected in 10 women (urinary albumin excretion (UAE) < 300 mg/24h), their number increased to 14 (1st trimester -tr), and then to 18 (27.2%) by term. Those patients comprised Gr.1. Prior to conception macroalbuminuria was observed in 4 women, there were 6 such patients (9%) in the 2nd tr. By term their UAE was > 2 g/24h. Those patients were included in Gr.2. Forty two patients with normo-albuminuria throughout the pregnancy comprised Gr.3. **Results:** The patients' diabetes duration (yrs) was 11.0 \pm 6 (Gr.1), 18.7 \pm 7 (Gr.2) and 7.7 \pm 3 (Gr.3). We did not register statistically evident difference in HbA1c (%) between the groups: Gr.1 – 7.6 \pm 0.5 (1st tr); 7.2 \pm 0.4 (2nd tr); 7.2 \pm 0.4 (3rd tr); Gr.2 – 7.5 \pm 0.5, 7.0 \pm 0.6, 6.8 \pm 0.4, and Gr.3 – 7.4 \pm 0.3, 7.1 \pm 0.4, 7.0 \pm 0.2, respectively. The highest insulin requirements (U/kg) were observed in all the patients throughout the 3rd tr – Gr.1, 0.75 \pm 0.15; Gr.2, 0.68 \pm 0.04, Gr.3, 0.97 \pm 0.06. Proliferative retinopathy was found in ten Gr.1 patients (55.5%), two Gr.2 patients (33.3%), and two Gr.3 patients (4.7%). Four Gr.2 patients had proliferative retinopathy (66.6%). There was no retinopathy progression observed after delivery. In Gr.2 patients anti-hypertensive therapy was used from the 2nd tr. There were no intrauterine or perinatal deaths. Gestational week at delivery (GWD) was 37-39 in Gr.1. In 38.8% of cases Caesarean section was performed. Infants' birth weight (IBW) was 3100 \pm 450 gr. In Gr.2 GWD was 34-38. All the patients (100%) underwent Caesarean section, IBW- 2800 \pm 200g. Three newborns had respiratory distress syndrome. In Gr.3 GWD was 38-40, Caesarean section was performed in 14% of cases, IBW-3500 \pm 640g. In 8 newborns hypoglycemia was observed. **Conclusions:** When women with diabetic nephropathy were well-controlled during the pregnancy, renal function deterioration was not associated with retinopathy progression. Presence of macroalbuminuria may predict fetal hypotrophy, respiratory distress syndrome and pre-term delivery.

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BIRTHWEIGHT AND SUBSEQUENT GROWTH INTERACTION AMPLIFIES LOW BIRTH WEIGHT PHENOTYPE EFFECT ON BLOOD PRESSURE AND INSULIN RESISTANCE

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Background and Aims: Low birth weight is associated with an increased risk of insulin resistance, increased blood pressure and cardiovascular disease in adult life. We examined whether the extent of subsequent growth (ie higher adult weight) affected expression of this phenotype in disadvantaged urban South Africans.

Materials and Methods: Twenty year olds ($n = 132$) with birth weights either < 10 th centile (UFA) or between 25th and 75th centile (AFA) for gestational age at full-term, had anthropometry, blood pressure, lipids and glucose tolerance measured. Insulin sensitivity (IR) and secretion (B) were assessed using the HOMA model. Gender specific median BMI for all subjects was used to define subsequent growth providing 4 groups: UFA-BMD ($n = 43$) UFA-AMD ($n = 25$) AFA-BMD ($n = 23$) and AFA-AMD ($n = 41$). Analysis of variance was used to assess group differences.

Results: Birth weights were lower in both UFA than both AFA groups (2.3 \pm 0.2 vs 3.1 \pm 0.2 kg, $p < 0.03$). Maternal BMI did not differ within birth weight groups but was lower in UFA-BMD than the two AFA groups ($p < 0.01$). Subjects current BMI was 19.5 \pm 1.5, 24.6 \pm 3.0, 20.2 \pm 2.0 and 25.2 \pm 4.8 kg/m² in UFA-BMD, UFA-AMD, AFA-BMD and AFA-AMD groups ($p < 0.01$ between BMI groups). Systolic BP was higher in UFA-AMD than UFA-BMD, AFA-BMD and AFA-AMD (130 \pm 16 vs 124 \pm 11, 119 \pm 10, 124 \pm 12 mmHg, $p < 0.02$). HOMA IR was higher in UFA-AMD (2.5 \pm 1.2) than UFA-BMD (1.6 \pm 1.0) and AFA-BMD (1.5 \pm 0.8) groups ($p < 0.01$). Triglycerides were higher in UFA-AMD than UFA-BMD and AFA-AMD (0.9 \pm 0.6 vs 0.6 \pm 0.2, 0.7 \pm 0.2 mmol/l, $p < 0.02$). Maternal smoking, education and housing density at birth, current housing density and level of education did not differ between groups. Diastolic BP, glucose levels (fasting and post OGTT), HOMA B and cholesterol were also not different between groups.

Conclusions: These data indicate that the interaction between birth weight and subsequent growth defined by adult BMI amplifies the expression of the chronic disease phenotype associated with low birth weight, at least in this cohort.

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CAN ACTIVIN A AND INHIBIN A PREDICT DEVELOPMENT OF PRE-ECLAMPSIA IN WOMEN WITH TYPE 1 DIABETES?

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Background and Aims: It has been shown that maternal serum concentrations of activin A and inhibin A are elevated in women without diabetes subsequently developing pre-eclampsia. Our aim was to determine if activin A and inhibin A can predict development of pre-eclampsia in type 1 diabetes. **Materials and Methods:** In a prospective study maternal serum was analysed for activin A and inhibin A in 115 type 1 diabetic women at 10,14,20,28 and 34 weeks of gestation. Twin deliveries were excluded. Pre-eclampsia was defined as BP >140/90 and proteinuria >300 mg/24h later than 20 weeks. **Results:** Fourteen women developed pre-eclampsia (week 30-37) and 101 did not. The two groups were comparable regarding age, BMI and diabetes duration. Booking BP was 130(10)/79(5), 114(11)/68(8) mmHg ($p<0.001$) and 6(43%); 6(6%) ($p<0.001$) had urinary albumin excretion ≥ 30 mg/24h. HbA1c at gestation was 8.0(0.9); 7.5(1.2) % (NS). Four(29%); 3(3%) ($p<0.01$) delivered preterm <34 weeks.

Table. Maternal serum concentrations of activin A and inhibin A during pregnancy.

Weeks of gestation	Activin A (ng/ml)		Inhibin A (pg/ml)	
	Pre-eclampsia	No pre-eclampsia	Pre-eclampsia	No pre-eclampsia
Week 10	0.9 (0.5-1.6)	1.0 (0.4-5.5)	269 (132-484)	328 (124-902)
Week 14	1.4 (0.7-2.7)	1.2 (0.6-3.9)	251 (132-747)	231 (84-1381)
Week 21	2.0 (1.3-4.2)	1.5 (0.7-3.9)	285 (96-1421)	242 (99-829)
Week 28	5.4 (2.4-46.4)	4.6 (1.9-11.9)	769 (224-8220)	709 (260-2844)
Week 34	12.1 (4.4-92.0)	12.1 (4.4-39.9)	1441 (488-13024)	1653 (504-5192)

Values are medians and (range). Differences between the 2 groups were NS.

Conclusions: Maternal serum concentrations of activin A and inhibin A are not useful as predictors of pre-eclampsia in type 1 diabetes.

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WHO MISSES ROUTINE REVIEW? - INFORMATION FROM A DISTRICT DIABETES REGISTER

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Background and Aims: Following recommendations from the Scottish Intercollegiate Guidelines Network (SIGN), a district diabetes register was established in Ayrshire and Arran, Scotland. This Register has been analysed to give information on the population not accessing routine diabetes care. **Materials and Methods:** Information for the register has been collected from the Diabetes Care System used in the Hospital Diabetes Clinics, General Practices, biochemistry laboratory, accredited optometrists, and the Social Work Department register of blind and partially sighted. The local population with diabetes who had not had HbA1c measured or dilated fundoscopy undertaken in the previous year (1999-2000) were identified. A number of demographic factors including age, socio-economic status (denoted by the Carstairs DEPCAT score derived from postcode) along with sex were investigated for those patients not accessing the diabetes service. A third group of diabetic patients who smoked were identified and assessed by socio-economic status to allow comparison between the diabetes register population and other populations for whom this type of analysis has been undertaken. **Results:** Of the 7573 patients, 10.5% (796) had no record of HbA1c and of the 7498 patients eligible for fundoscopy 24.4% (1834) had not had dilated fundoscopy performed. Patients under 30 years ($p<0.01$) and over 80 years ($p<0.01$) were more likely to miss HbA1c check and fundoscopy. Patients with DEPCAT score 1&2 ($p<0.01$) plus 6 ($p<0.01$) were more likely to miss HbA1c check and fundoscopy. There was no significant difference in uptake by gender. The register demonstrated an increasing prevalence of smoking with increased deprivation. **Conclusion:** The data held on the Ayrshire and Arran Diabetes Register suggests that age and socio-economic status influences the uptake of a routine diabetic service. Planners of health care need to give consideration to the requirements of different age and socio-economic groups.

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Improvement of the quality of diabetes care - JEVIN, a population-based survey with 10-years-follow-up: 1989/1990 - 1999/2000

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Diabetes m.	Type 1			Type 2		
	1989/90	1994/95	1999/2000	1989/90	1994/95	1999/2000
No. (n)	131	127	114	59	117	147
BGSt (n/d) ¹	1 (0-28)	25 (0-49)*	28 (0-70)*	0 (0-21)	14 (0-35)*	21 (0-46)*
ICT (%) ²	5.3	80.3*	94.7*	3.4*	30.8*	48.3*
TTP (%) ³	0	73.2*	87.7*	0	89.7*	96.6*
Rel. HbA1c	1.52±0.3	1.65±0.4*	1.48±0.3*	1.78±0.3	1.75±0.4	1.47±0.2*
Hypogly. ⁴	0.08	0.13	0.16	0.02	0.04	0.01
Coma ⁵	0.015	0.018	0.018	0	0.008	0

There was no increase, neither in patients with type 1 nor type 2 diabetes, in the prevalence of long-term complications (nephropathy, retinopathy, neuropathy).

Conclusions: During the last decade there was a substantial improvement in the quality of diabetes control. The broad implementation of specialised care, structured TTPs, intensified insulin therapy and blood glucose self-monitoring for patients with type 1 and 2 diabetes mellitus seems to be major corner stones for the improvement of the quality of diabetes care.

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Quality of care of elderly type 2 diabetics in general practice: results from the French Adage 65+ Study
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Aims and Methods: The national study Adage 65+ was performed by 456 French general practitioners to analyze the state of health and quality of care of 1349 type 2 diabetics over the age of 65 years receiving oral antidiabetic drugs. Each practitioner was asked to fill a questionnaire and data were analyzed by a single statistical center.

Results: This study concerned patients with a mean age of 71.6 ± 5.7 years (56% male - 44% female) and an average duration of the disease of 11 years. A high prevalence of cardiovascular risk factors is noted: smoking (36%), obesity or excessweight (79%), mostly android (63% of men, 75% of women), hypertension (80%) or drug treated hyperlipidemia (58%). Metabolic control remains inadequate: fasting glucose 153 ± 43 mg/dl, HbA1c $7.5 \pm 1.5\%$ (the latter was only determined among 65% subjects). Similar findings are observed for other risk factors: blood pressure is controlled in only 20% of patients, although over 2/3 of patients receive antihypertensive drugs. LDL cholesterol is only occasionally determined. Degenerative complications are surprisingly uncommon: coronaropathy 17%, arteritis 15%, retinopathy 13%, neuropathy 7%, diabetic foot 5%, nephropathy 5% (while urinalysis is performed only among 27% patients), suggesting a poor detection of complications. Finally, multidisciplinary diabetes care seems improved when compared to previous French studies: 92% of patients per year are referred to ophthalmologists, 71% to cardiologists but only 23% to diabetologists.

Conclusion: Elderly type 2 diabetics present with clinical characteristics similar to middle age diabetics. Since type 2 diabetes is a complex disorder, our study emphasizes the need for early and multispecialized overall care of type 2 diabetic patients regardless their age.

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A COMPARISON OF 2 PAEDIATRIC AUDITS IN YORKSHIRE, UK
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Background and Aims: In 1992 an audit of service delivery for paediatric diabetes recommended improvements in the ranges of hospital support and delivery of care from a trained paediatrician. In 1997 a further audit established these recommendations had been implemented. This study aimed to investigate whether improved health care in clinics across the Yorkshire region was actually reflected in improved diabetic control as measured by HbA1c levels of the patients.

Materials and Methods: Demographic and clinical details were collected by a single data collector on a cross section of 798 children (0-18 years) attending 24 clinics in 1992 and 650 children at 15 clinics in 1997. Mean HbA1c levels and insulin dose/kg were compared between audits.

Results: There was no difference in age distribution either at diagnosis or at the time of the audit between 1992 and 1997 with 32% in 1992 and 37% in 1997 being under 5 years of age at diagnosis. There was a significant reduction in HbA1c of 1% ($P < 0.01$) from 9.8% to 8.8%. There was no significant difference in the average daily dose of insulin/kg between audits. In 1997, the mean dose increased from 0.77 units/kg/day in 0-4 year olds to 1.16 units/kg/day in 10-15 year olds.

Conclusions: This study has demonstrated that more specialised and focused delivery of care appears to improve diabetic control in a large clinic population representative of the spectrum of diabetes.

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STAGED DIABETES MANAGEMENT VERSUS TRADITIONAL APPROACH IN THE TREATMENT OF DIABETES IN ST-PETERSBURG.

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Background and Aims: Staged Diabetes Management (SDM) represents a systemic approach to the diabetes management and its efficacy has been proved in countries with different health care systems. To implement the SDM in St-Petersburg we performed its adaptation to local health care and pharmacy system. The aim of the present study was to compare the efficacy of SDM with the traditional treatment regimen of DM in selected out-patient diabetes clinics. **Materials and Methods:** 110 patients with DM (type 1 - 50, type 2 - 60) were recruited in 6 out-patient centres. The randomisation (2:1) was performed per centre: the SDM group - 80 pts and control group - 30 pts. The physicians, randomised for SDM, contrary to control group were specially trained for SDM algorithms. All patients were equally equipped with streeps for self-control and passed a standard education course according to local practice. The efficacy criteria were: fasting glycemia, HbA1 (N:5-7%), total insulin dose (U/kg/day) or oral hypoglycemic agents (OHA) requirement, intensity of treatment (number of visits per 1 pt per 6 months), and frequency of hypoglycemias. All tests were collected at baseline, at 3 and 6 months of the follow-up. **Results:** The median of fasting blood glucose decreased in both groups: SDM - $9.4-7.0-7.0$ mmol/l; control - $8.85-7.0-7.3$ mmol/l (for SDM and control $p < 0.05$). HbA1 decreased in SDM group ($8.6 - 8.1 - 7.4\%$, $p < 0.05$), whereas in control group it remained unchanged ($7.8 - 8.2 - 7.8\%$). Intensity of treatment in type 1 DM was higher in SDM group (7.3 vs 5.2 visits/1pt/6 months in control group, $p < 0.05$). The total insulin dose and frequency of hypoglycemias did not changed significantly in both groups. Intensity of treatment in type 2 DM was higher in controls (7.6 vs 6.8 visits/1pt/6 months). At the same time, in 24% patients with type 2 DM of SDM group the OHA doses were lowered. In control group there was no decrease of OHA dose and in 30% cases one OHA was changed to another without effect. **Conclusions:** The treatment according to modified SDM algorithms lead to significant improvement in glycemic control and was superior compared to traditional treatment. The improvement of treatment of DM in SDM group was predominantly achieved by optimisation of treatment schemes while increase of intensity of treatment was unfrequent.

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EFFICACY OF A STRUCTURED PATIENT MANAGEMENT SYSTEM FOR DIABETIC PATIENTS

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Background and Aims: Prospective analysis of the effects of a patient management system on cardio-vascular risk profile in diabetic patients. **Patients and Methods:** The structured system aims at optimization of glucose metabolism, blood pressure and lipid profile, dietary adaptation and cessation of smoking. Physicians are provided with guideline-based diagnostic and therapeutic recommendations. Every 3 months structured data are imported into a central database, and for each patient a separate analysis containing comments and alerts is generated. 3,949 diabetic patients from 202 primary care offices were included into the system. 2066 patients with an observation period of >6 months were divided into 2 groups: 696 normoalbuminuric patients (Group A) and 1370 patients with microalbuminuria (Group B; definition: no exclusion criteria, ≥ 20 mg/l in at least 2 of 3 samples of first morning obtained within one week, no positive result for protein in urinalysis, normal serum creatinine level). Basic data in Group A (B) were (mean \pm SD): age 44% (52%) male, age 63.0 ± 11.8 (63.0 ± 12.3) yrs, known diabetes duration 8.0 ± 8.7 (9.1 ± 9.8) yrs. **Results:** After 16.9 ± 8.0 (17.5 ± 8.5) months values compared to baseline values were: HbA1c $7.2 \pm 1.4\%$ vs. $7.5 \pm 1.5\%$, $p < 0.01$ ($7.3 \pm 1.4\%$ vs. $7.7 \pm 1.7\%$, $p < 0.0001$). RR_{syst} 141 ± 17 vs. 144 ± 19 mmHg, $p < 0.001$ (141 ± 17 vs. 146 ± 20 , $p < 0.0001$). RR_{diast} 81 ± 9 vs. 82 ± 10 , n.s. (80 ± 9 vs. 83 ± 11 , $p < 0.0001$). Total serum cholesterol 217 ± 38 vs. 223 ± 40 mg/dl, $p < 0.001$ (213 ± 44 vs. 222 ± 44 , $p < 0.0001$). Triglycerides 170 ± 105 vs. 179 ± 125 mg/dl, n.s. (179 ± 116 vs. 201 ± 145 , $p < 0.0001$). At the end of the intervention period 69% (37%) were normoalbuminuric, 23% (48%) microalbuminuric, 6% (11%) macroalbuminuric, and 2% (4%) showed an increased serum creatinine. **Conclusion:** This system implementing methods of quality management into routine diabetes care showed a significant reduction of the cardiovascular risk profile.

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CARDIOVASCULAR MORBIDITY AND RESOURCE CONSUMPTION: AN ASSESSMENT OF THEIR AVOIDABILITY IN TYPE 2 DIABETES.

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Background and Aims: In the framework of a nation-wide outcomes research program in type 2 diabetes, we assessed the avoidability of cardiovascular (CV) events related to the implementation of the existing guidelines for the management of hypertension and dyslipidemia. The excess of resource utilisation related to the development of CV events was also estimated.

Materials and Methods: Type 2 diabetic patients younger than 75 years and free from major cardiovascular diseases were included in this study. By applying the Framingham equations, we estimated their 10 year CV risk and then calculated the CV risk reduction that could be achieved by pursuing different therapeutic targets. Furthermore, in 821 patients with a history of CV disease, resource utilisation in the previous year was evaluated in terms of disability days, hospitalisation days, number of clinical visits and diagnostic procedures.

Results: Overall, 1689 patients were eligible for this study. The mean risk of developing a CV event during 10 years was $18.9 \pm 8.7\%$. The number of avoidable events during 10 years was strongly related to patients' baseline risk. By decreasing systolic blood pressure to 130 mmHg, the number of events avoided (per 1000 treated subjects) would be 6 in low risk patients (risk <15%) and 34 in high risk ones (risk >30%). Similarly, by decreasing total cholesterol levels to 150 mg/dl, 25 events would be avoided in low risk patients and 76 in high risk ones. The maximum risk reduction rate, corresponding to 38% (105 avoided events/1000 treated subjects), would be attained by aggressively treating both risk factors. If applied to the Italian population, these figures would translate in over 40.000 CV events avoided during 10 years. Excess resource consumption in patients with a history of CV disease was the following (per 1000 patients/year): 444 hospitalisation days, 6850 disability days, 2078 clinical visits, and 2112 diagnostic procedures.

Conclusions: A strict control of blood pressure and hypercholesterolemia, in agreement with existing guidelines, could lead to a substantial decrease in morbidity, social and economical costs related to diabetes.

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CARDIOVASCULAR THERAPIES IN 106 DIABETES CLINICS: A PHARMACO-EPIDEMIOLOGICAL ANALYSIS.

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BACKGROUND: Several studies have shown how guidelines are poorly implemented in clinical practice. Out-of-pocket cost for medication, load of daily tablets, tightened drug-budget control, and physicians' prescription attitudes are some of the possible explaining factors. **AIM:** To evaluate how primary e secondary prevention of cardiovascular (CV) disease has been carried out in over time in a large sample of Italian diabetic clinics. **MATERIALS AND METHODS:** The source of data was the database of the DAI study at 6-month and 12-month follow-ups. The DAI Study is an observational study on macroangiopathy in type 2 diabetic which randomly selected 24056 patients from 201 Italian diabetic clinics. Data on treatment are being collected every 6 months. After a detailed descriptive evaluation in several subsets of patients, certain cost were calculated in 106 clinics. **RESULTS:** On average, in primary prevention 11.5% of patients were on antiplatelets, 23% on lipid-lowering and 57% on antihypertensive therapies. In secondary prevention 46% of patients were on antiplatelets and 33% on lipid-lowering therapies. In the period of time Jan 1999-Jan 2000 a fair increase in the proportion of treated patients was recorded, especially for statins (+24%). This trend was particularly apparent in subjects who developed cardiovascular events in the same period of time. The mean expense for cardiovascular treatment per patient was 236 Euros per year. An appropriate link between CV risk, calculated by using Framingham score equation, and drug expenditure was found. However, the ratio between number of treatments and number of patients who would need it, in every center, showed that drug utilisation was very varied among clinics. **CONCLUSIONS:** As regards effective treatment to prevent CV disease in diabetic patients, a positive trend over 1 year was found in this representative sample of diabetic clinics. Recent cardiovascular events sensitize doctors to start effective therapies. Prescription attitudes are anyway varied among clinics, there is a sort of here-and-there pattern of prescription. On the whole, treatment of cardiovascular risk factors in diabetic patients warrant further efforts.

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Why is the cardiovascular risk profile in type 2 diabetes so hard to improve?

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Background and Aims: Improving the cardiovascular risk profile is an important aspect in the treatment of type 2 diabetes as 70% of these patients die of cardiovascular disease. A very important key to success in improving the risk profile is motivation of the patient. The latter is highly dependent on expectations regarding the improved Quality of Life (QoL) that may be felt as a consequence of the effects from changes in behaviour or treatment. We studied the relationship between cardiovascular risk factors and QoL.

Materials and Methods: In the shared care diabetes project ZODIAC (Zwolle Outpatient Diabetes project Integrating Available Care) QoL is assessed using the Rand-36, a generic questionnaire. For this cross-sectional study 1012 questionnaires from 1155 patients were included; non-parametric tests were used for analyses.

Results: Mean HbA1c was 7.5%, total cholesterol 5.7 mmol/L, blood pressure 155/84 mmHg, and 19% of the patients smoked. Only one (health change) out of 9 scales of the Rand-36 showed a significant negative relationship with HbA1c ($p=0.005$). No significant relationship was found between any of the scales and total cholesterol, nor with blood pressure or smoking.

Conclusions: An important explanation of the fact that it is difficult to improve the cardiovascular risk profile in type 2 diabetic patients is that there is no direct relationship between the degree of regulation of these risk factors and QoL. Acknowledgement of this fact by caregivers might lead to efforts to motivate patients to change behaviour in other ways than by highlighting possible changes in QoL.

PS 69 Education

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An innovative experience of nutritional education at primary school

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Background and Aims: weight excess and obesity constitute a growing widespread health problem. The present action aimed to experiment an educational program for nutrition at primary school for 9-11 yrs children. **Materials and Methods:** 5 practitioners, 10 nurses from the school health promotion and 11 teachers followed a training program (2x4 hrs): nutritional balance and repartition, common health messages and pedagogic tools. The education phase was conducted (Jan-Jun 2000) by the trained teachers in 11 classes. Body Mass Index was determined in 198 children (Group E) before (t0) and at the end (t1) of the education phase, children perceptions on health and food were assessed by the means of a 10 items questionnaire. The same data were collected at t0 and t1 in a non educated control group (Group C, n=191) from the same schools. Groups E and C were not different concerning age and sex (M: 46% ; F: 54%). Results were analysed using chi-2 and variance analysis. **Results:** at t0, weight excess (BMI > 90th percentile) was present in 31.8% (n=63) children in group E and 30.9% (n=59) in group C, obesity (BMI > 97th percentile) in 20.7 % (E, n=41) and 19.4 % (C, n=37). Children perceptions were identical at t0 in the 2 groups : healthy role of dairy products, fruits or vegetables, and importance of breakfast were correctly reported (>80 %), but errors predisposing to obesity were frequent : 80 % thought that a large amount of meat is necessary, up to 20 % did not perceive the dangerous effect of sources of non-visible lipids, 50 % thought that sugar and sweetened products are indispensable to growth. At t1, weight excess was present in 29.6% (E : 33.3%, n=66; C : 25.7%, n=49, ns), obesity in 16.2% (E : 17.2%, n=34; C : 15.2%, n=29, ns). Overall, there was no significant differences at t1 vs t0, albeit the numbers of obese boys tended to reduce in group E (9.4%, n=9 vs 17.7%, n=17). Amelioration of perceptions was noted in the 2 groups at t1, but was better in group E. **Conclusion.** Introduction of nutritional education at school is feasible, provided that action is prepared in the context of a structured network of nutrition experts, school health professionals, and institutional covering of a designed project. The effect of such an action is not obvious as regards to anthropometric data at the end of education period, but perceptions of educated children seem to improve. An additional evaluation has to be done at one year (t2, June 2001), and the actual impact on behavioural changes needs to be assessed.

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THE DETMOLD DIABETIC EMERGENCIES PROJECT - A POPULATION-BASED INTERVENTION STUDY TO IMPROVE THE QUALITY OF PREHOSPITAL MANAGEMENT OF DIABETIC EMERGENCIES

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Aims: Studies have revealed deficits in the pre-hospital management of diabetic emergencies. An intervention study was performed to evaluate prospectively the effects of diabetological training of the emergency medical team, blood glucose screening of all emergency patients and standardized therapy of diabetic emergencies. **Methods:** After initial diabetological training of all emergency physicians and emergency medical technicians, a standardized protocol was introduced for the pre-hospital emergency therapy of severe hypoglycaemia and diabetic coma in a German emergency medical service district with 180,000 inhabitants in the period from 1997-2000. For sensitive detection of diabetic emergencies, a rapid blood glucose test was performed in all emergency patients with the exception of small children, resuscitations and deaths. Indicators of structural, process and treatment quality before and after intervention were compared. **Results:** A rapid blood glucose test was performed in 6631 (85%) of all 7804 emergencies. The prevalence of acute diabetic complications was 3.1%. 213 cases of severe hypoglycaemia and 29 cases of diabetic coma were recorded. Compared with a retrospective analysis of the 204 diabetic emergencies of the period 1993-1996 in the same district there was a continuous increase in diabetic emergencies. The training of the emergency team led to a significant improvement in the quality of treatment. In hypoglycaemia larger volumes of intravenous 40% glucose were administered (50±20 ml vs. 28±20ml; p<0.001). In 50 diabetic patients with sulphonylurea-induced hypoglycaemia the mandatory additional glucose infusions and hospitalization for further observation reduced mortality from 4.9% to currently 0%. Insulin-treated patients well educated about diabetes were more often treated at the emergency scene only after severe hypoglycaemia (25% vs. 8%), hospitalization was avoided without complications. For the first time emergency medical technicians independently treated hypoglycaemia before the arrival of the emergency physician. **Conclusions:** The diabetological training of the emergency team is effective and efficient, it improves the quality of treatment, the prognosis and the economic variables of diabetic emergencies.

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KNOWLEDGE OF THE FINDINGS OF THE UK PROSPECTIVE DIABETES STUDY (UKPDS) AMONGST UK GENERAL PRACTITIONERS

A.M.Emslie-Smith, J.M.Evans, G.P.Leese and A.D.Morris for the DARTS/MEMO Collaboration, Ninewells Hospital & Medical School, Dundee, UK. **Background and Aims:** To assess UK General Practitioners' level of knowledge of the findings of the UKPDS. **Materials and Methods:** 25 questions, based on UKPDS findings, were posted, in June 2000, to all 109 General Practitioners, and to two Diabetologists in the city of Dundee, UK. **Results:** 61 questionnaires (56%) were returned. Mean score = 25.6% (range 0-68%). There was no significant difference in mean score between those in Practices of a certain size, Training Practices or Practices with dedicated diabetes clinics, nor between GPs who subscribed to the *British Medical Journal*, were members of the Royal College of General Practice or who were GP Trainers, and those who were not. GPs who were actively involved in diabetes care in their Practice (n=30, mean score 31.4%) scored better (p=0.01) than those who were not (n=31, mean score 19.5%). There was no significant difference in correct answers to questions relating to glycaemic control (n=12, average % correct 26.0%) compared with those relating to blood pressure control (n=11, average % correct 24.9%). 37.7% (46.7% if active in diabetes care, 29.0% if not) knew that UKPDS was restricted to Type 2 diabetes. Questions on different drug treatment approaches showed correct knowledge in 18.0% (25.0% if active, 11.3% if not) of respondents for glycaemic therapy and 39.3% (46.7% if active, 32.3% if not) for blood pressure therapy. Correct knowledge of targets for control of glycaemia and blood pressure, developed as a result of UKPDS, was shown by 34.4% (46.7% if active, 22.6% if not) and 47.5% (63.3% if active, 32.3% if not) respectively. Both Diabetologists answered 24 questions correctly (96%). **Conclusions:** General Practitioners provide most Type 2 diabetes care in UK. However, their level of knowledge of the UKPDS findings appears to be low. The significant difference between those active in diabetes care and others, demonstrates the trend towards sub-specialisation within General Practice. If evidence-based medicine is to be practised, new methods must be found to more effectively educate GPs of trial findings.

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UTILISING MICROALBUMINURIA AS A SPECIFIC RISK MARKER HAS AN ADDITIONAL TREATMENT EFFECT IN TYPE 2 DIABETIC PATIENTS

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Background and Aims: To assess the effect of focusing on microalbuminuria in a multifaceted intervention directed at general practitioners to improve care of type 2 diabetic patients.

Material and Methods: 474 general practitioners participated in a pragmatic, open, controlled trial with randomisation of practices to structured personal care (intervention group) or routine care (control group). 874 (90.1%) of 970 patients aged 40+ years diagnosed with diabetes in 1989-91 and surviving until 6-year follow-up. Intervention included regular follow-up and individualised goal-setting supported by prompting of doctors, clinical guidelines, feedback and continuing medical education. In the annual patient reports sent to doctors no specific advice concerning treatment were given except for patients with microalbuminuria for whom doctors were asked to consider treatment of even slight hypertension. Urinary Albumin Concentration (UAC) in a freshly voided morning urine sample was analysed centrally using a RIA at the diabetes diagnosis. The patients (n) of the intervention and control group, respectively, were: normoalbuminuric (292/275), microalbuminuric (127/106) and proteinuric (19/19), defined as a UAC (mg/l) of <15, 15-200 and >200, respectively. **Results:** After 6-years of follow-up the systolic blood pressure (medians, mmHg) in the intervention and control group, respectively, were 145 vs. 150 (p<0.0001, Wilcoxon test), while the diastolic blood pressure were 80 vs. 84 (p=0.23). Stratified by normoalbuminuria, microalbuminuria and proteinuria at baseline, the systolic blood pressure in the intervention and control group, respectively, at follow-up were 146.5 vs. 150 (p=0.015), 145 vs. 155 (p=0.003), 150 vs. 160 (p=0.12) in the three groups, while the diastolic blood pressure were 80 vs. 81 (p=0.71), 80 vs. 85 (p=0.060), 85 vs. 85 (p=0.36).

Conclusions: Judged from the effect of this multifaceted intervention on blood pressure of type 2 diabetic patients, patient-specific written medication-advice to doctors of patients with microalbuminuria has an additional beneficial and clinical important treatment effect.

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Comparative analysis of conventional and adaptive computer-based interactive hypoglycaemia education programs

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Objective: Conventional computer-based education programs do not take into account interpersonal differences among patients such as different skills using computers or different diabetes knowledge. Our aim was therefore to overcome this disadvantage by creating an adaptive, interactive computer based training program which can be personalised to the patients' specific needs and skills. To test whether this aim can be achieved we therefore compared a conventional with an adaptive computer-based hypoglycaemia education program with regard to parameters like increase in diabetes knowledge or user friendliness. **Research Design and Methods:** The patients were confronted with a hypoglycaemic situation and asked to overcome this situation by choosing the most helpful actions from a variety of opportunities provided by the computer program. 120 randomised diabetic patients were enrolled in this study. First, we compared the results (number of mistakes) obtained with the conventional and the adaptive computer program. Second, we determined the time patients needed to finish the different exercises. Third, the user friendliness of the two programs was evaluated by means of a questionnaire. Differences among the two groups were tested for statistical significance using the Mann-Whitney-U-Test for independent groups. A p value <0.05 was regarded as statistically significant. **Results:** Patients using the adaptive computer-based hypoglycaemia education program had significantly better results ($p=0.037$) as compared to those using the conventional training program. Furthermore, the user friendliness of the adaptive computer program was rated as significantly better ($p=0.015$) by the patients. **Conclusions:** The adaptive, interactive computer-based hypoglycaemia education program is more effective and better accepted compared to a conventional training program. Therefore, this adaptive education program might be a helpful adjunct to the current hypoglycaemia education of diabetic patients.

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Structured teaching and treatment of elderly patients with type-2 diabetes mellitus and impaired cognitive function- the DikoL-intervention trial

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Structured teaching and treatment programmes (TTP) should be offered to all patients with diabetes. After participation in a TTP, 50% of the elderly patients are still inadequately trained (Schiel et al., Diab Stoffw 2000; 9: 227-233). They often need third party assistance leading to impaired quality of life and increased welfare costs. Thus, it was the aim of this intervention trial to develop a structured TTP for elderly patients with impaired cognitive function. **Methods:** 106 patients with type-2 diabetes older than 55 participating in a TTP for conventional insulin therapy in 1999 were examined. Patients with less than 91 IQ-points were randomised: They either took part in the TTP according to Berger et al. (standard group) or in the special adapted DikoL-TTP (no pathophysiology, urinary- instead of bloodglucose-self-monitoring, more practical exercise). **Results:** Group A/B: $n=35/34$, age $67.6 \pm 8.9/70.7 \pm 8.2$, $p=0.14$, diabetes duration $11.6 \pm 8.3/11.1 \pm 7.4$ ys., $p=0.80$, HbA1c $10.3 \pm 2.0/10.7 \pm 1.8\%$ [HPLC, Diamat® NR 4.5-6.3%], $p=0.33$, cognitive function $81.5 \pm 5.1/78.8 \pm 6.7$ IQ-points, $p=0.07$. After the TTP and ½ year later patients' knowledge and ability of diabetes-self-management were tested.

Baseline	Standard(n=30)	DikoL (n=26)	p-value
Knowledge (points)	10.9±2.6	12.2±2.7	0.08
Handling (points)	14.2±3.3	15.9±2.5	0.17
HbA1c (%)	10.4±2.1	10.4±1.5	0.90
Re-examination			
Knowledge (points)	8.8±5.0	8.7±5.1	0.97
Handling (points)	12.4±4.1	15.9±2.5	0.001
HbA1c (%)	8.4±1.4	8.5±1.3	0.77

There were no differences in incidences of hypoglycaemia, coma and foot ulcers at re-examination. **Conclusions:** Elderly patients with impaired cognitive function should take part in a special adapted TTP. This shows equal, concerning "diabetes-self-management", even more efficiency with better acceptance of the patients.

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QUALITY OF LIFE FOLLOWING AN EDUCATIONAL INTERVENTION

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Background and Aims: Quality of life (QoL) is an important outcome measure in diabetes care. This study was aimed to examine the long-term effects of a team educational intervention on the patients' quality of life.

Materials and Methods: 395 patients (212 females, aged 39.2 ± 15.1 yrs, suffering from diabetes for 11.4 ± 8.7 yrs, treated with insulin in 89% cases, having baseline HbA1c values $8.6\% \pm 2.3$, educated at primary level in 10.4%, secondary in 66.6% and university in 23%), were included in a 5-day educational course, structured as a small-group educational and psychological workshop. The groups were invited for medical check-ups and educational refreshments at 3-month intervals, and assessed regarding their QoL prior to the course, and after follow-up periods of 3 and 18 months. The WHOQoL-BREF, a 26-item generic QoL instrument was used. The instrument covers four QoL domains: Physical, Psychological, Social relations and Environment. Paired t-tests and correlations were employed to compare QoL indicators at different assessment points.

Results: When compared with baseline indicators, the patients' QoL indicators improved after 3 months with regard to their overall QoL ($t=-4.3$ $p=0.000$), satisfaction with health ($t=-8.5$ $p=0.000$), Physical domain ($t=-6.44$ $p=0.000$), Psychological domain ($t=-4.29$ $p=0.000$), Social domain ($t=-3.25$ $p=0.001$) and Environment ($t=-4.69$ $p=0.000$). The improved QoL remained stable after 18 months for all the four domains, while the subjective evaluations of overall QoL and general health further improved. The indicators of metabolic control were improved after 3 months to $8.0\% \pm 1.8$, and $7.8\% \pm 1.6$ after 18 months. The associations between glycemic control as measured by HbA1c and QoL were shown to be significant for physical and psychological domains at baseline ($r=-0.13$ $r=-0.11$), for physical, psychological and social domains at the 3-month follow-up period ($r=-0.11$ $r=-0.17$ $r=-0.12$) and for physical domain after a 18-month follow-up period ($r=-0.29$).

Conclusions: A team educational intervention followed by regular check-ups has long-term positive effects on the patients' quality of life.

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PERCEPTION OF RETINOPATHY AND SCREENING PROCEDURES AMONG DIABETIC PEOPLE

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Background and Aims: Managing chronic diseases requires that patients have correct perceptions of their condition and how to prevent complications through appropriate health beliefs, lifestyle changes and control of health practices. Diabetic retinopathy (DR) is likely to occur during a patient's lifetime and regular screening prevents visual loss. This study was designed to verify how patients perceive DR, its screening and their own role in preventing blindness. **Methods:** A questionnaire was administered to 258 consecutive patients after screening for DR, according to the European Field Guide-Book procedure, in Turin ($n=130$) and Wales ($n=128$, Group W). All Welsh patients and 70 in Turin (T1) were on standard medical care and education at their clinic or general practitioner, whereas the other 60 in Turin (T2) were on a permanent programme of diabetes care delivery through group tutoring. Statistical analysis was carried out first by comparing T2 patients with all those on standard medical care (T1 and W) and then the latter with each other. Comparisons were done by chi-square test on a 2x2 table. **Results:** Diabetes may damage the eyes according to 100% and 84% of patients in groups T2 and T1, respectively, and 50% in Wales ($p<0.01$ vs T1 and T2). DR had been heard of by 100% (T2), 67% (T1) and 48% (W) ($p<0.01$ vs T1 and T2). In group T2, 82% of patients could give a meaningful description of DR, though only 17% could use correctly the word "retina", but only 18% and 16% in groups T1 and W ($p<0.001$ vs T2); these patients either did not know the word "retina" or believed it was outside the eye (e.g. it was the whole eye or even the screening operator or the camera). In groups T1 and W, respectively 57% and 47% of patients believed they could not help with eye care, whereas 78% in T2 replied they should control their diabetes and 20% that their eyes should be checked regularly ($p<0.001$). Regarding reasons for screening, 100% of patients in group T2 answered "prevention and checks", against 61% in T1 ($p<0.001$ vs T2) and only 9% in W ($p<0.001$ vs T1). In groups T1 and W, 33% and 37% said that they did not know why they were being screened while all T2 patients did know. **Conclusions:** These preliminary data suggest that correct health perception and internal control mechanisms may be activated by permanent interactive education much more effectively than through information given during standard consultations.

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Socioeconomic Aspects of Diabetes

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Economic consequences of controlling both HbA1c and Post-Prandial Glucose in type 2 diabetes

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Background and Aims: Recent studies report correlation of the 2-hour postprandial glucose level (PPG) with the risk of cardiovascular disease. To explore the economic implications of controlling both HbA1c and PPG, we developed a short-term model that examines the efficiency of attaining this treatment goal in patients with type 2 diabetes.

Materials and Methods: A model of glycemic control, therapeutic decisions, and associated costs from the perspective of a comprehensive payer was developed using a Markov process. This process allows movement between various clinical states: glycemic control defined as control of either PPG or HbA1c or both, lack of control, and death. American Diabetes Association guidelines were considered for treatment goals or to take an action in relation to HbA1c. Visit rates were estimated based on guidelines. Costs are reported in 2000 US dollars discounted at 3%. Analyses were carried out comparing nateglinide to metformin based on the results of a randomized clinical trial.

Results: Simulation over a three-year time frame of a cohort of 10,000 drug-naïve diabetic patients starting with HbA1c ≥ 8.0 , showed that those starting on nateglinide would attain 21 months with dual control compared to 18 months for those starting on metformin. Total savings of USD \$295 with incremental savings of USD \$122 per month with dual control were projected on nateglinide treatment, mainly resulting from fewer visits and treatment changes. This finding was sensitive to the price of the drug and the starting HbA1c.

Conclusions: Achieving dual glycemic control can be more efficiently attained by starting patients on nateglinide than on metformin and this can yield savings in the costs of managing patients with type 2 diabetes.

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T¹ARDIS: The economic impacts of Type 2 Diabetes on the individual and their carer are far reaching

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Background and Aims: Type 2 diabetes is a life-long commitment for patient and family. The aim of the T¹ARDIS (Type 2 diabetes: Accounting for a major Resource Demand In Society) survey was to estimate the total cost of care for different groups of people with type 2 diabetes by assessing all direct and indirect costs and the intangible costs or quality of life (1,2,3). The focus is *the person* with diabetes and their carer rather than the cost of diabetes *per se*.

Material and Methods: A cross-sectional postal survey of a random sample of 3000 people with type 2 diabetes and their carers in four centres with diabetes registers was undertaken. Personal expenditure by patients and their carers, hours of informal care, health-related quality of life, the impact on the carer and levels of state benefits received were reported.

Results: 1578 patients and 500 carers responded to the survey. Mean personal expenditure for the individual with a carer was significant and exceeded £500 per year. Microvascular and/or macrovascular complications increased personal expenditure over 3-fold and doubled the likelihood of having a carer. Almost half the patients reported problems of mobility or self-care yet few (9%) accessed Social Services. One fifth of carers of patients with long-term complications provided over 60 hours of dedicated caring per week. Nearly two thirds of carers reported emotional, financial and physical strain. Over 70% of patients and carers reported receiving no state benefits.

Conclusions: The impact of type 2 diabetes is frequently viewed in terms of the costs to the NHS. T¹ARDIS has shown the pervasive impact of the condition on individuals and their carers. Complications exacerbate this impact. Resources and systems must be set in place to prevent complications, thereby improving the lives of people with type 2 diabetes, reducing the strain on their carers and potentially reducing the impact on the NHS. The diabetes National Service Frameworks in the UK expected later in 2001 may provide such an opportunity.

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QUALITY OF LIFE IN TYPE 2 DIABETES: THE CODE-2 EXPERIENCE. R. Mera, A.W. Bakst, and M. Auland. SmithKline Beecham, Collegeville, PA.

Background and Aims: The Cost of Diabetes in Europe-Type 2 (CODE-2) was a prevalence-based study designed to quantify independent predictors of health utility scores (economic and humanistic costs) as derived by the EuroQol, a self-administered questionnaire. **Materials and Methods:** Data were collected from 4189 patients with Type 2 diabetes (T2D) from Belgium, Italy, The Netherlands, Spain, and Sweden. **Results:** Derived EuroQol scores for patients with macrovascular and microvascular complications were analysed (uni = univariate, multi = multivariate). **Conclusions:** Independent predictors of quality of life, in order of importance, are: neuropathy, stroke, heart failure, retinopathy, dialysis, nephropathy and hypertension. These factors explain 77% of the variability of the EuroQol. Data from this study can be used for pharmacoeconomic assessment of new antidiabetic agents.

	EuroQol score	Uni, p	Multi, p
All T2D patients	0.69 (0.68-0.70)	-	-
No complications	0.78 (0.76-0.79)	0.000	-
Retinopathy	0.60 (0.58-0.62)	0.000	0.000
Blindness	0.55 (0.47-0.62)	0.000	0.228
Neuropathy	0.56 (0.54-0.58)	0.000	0.000
Microalbuminuria	0.62 (0.59-0.64)	0.000	0.156
Nephropathy	0.51 (0.47-0.56)	0.000	0.010
Dialysis	0.43 (0.34-0.50)	0.000	0.004
Angina	0.63 (0.61-0.66)	0.000	0.103
PTCA	0.65 (0.58-0.71)	0.174	0.568
Hypertension	0.67 (0.66-0.68)	0.000	0.010
Coronary heart failure	0.58 (0.54-0.61)	0.000	0.000
Myocardial infarction	0.67 (0.64-0.70)	0.049	0.096
Stroke	0.51 (0.47-0.56)	0.000	0.000
Micro-macro	0.56 (0.54-0.59)	0.000	-

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IMPACT OF CARDIOVASCULAR DISEASE ON MEDICAL CARE COSTS IN PERSONS WITH AND WITHOUT TYPE 2 DIABETES G.A. Nichols and J.B. Brown, Portland, Oregon, USA

Background and Aims: Diabetes (DM) and cardiovascular disease (CVD) greatly increase medical care costs. However, the impact of CVD on types of cost and on cost profiles has not been described in contemporary settings.

Materials and Methods: We compared the prevalence of CVD and 1999 medical care costs among persons with and without CVD in (1) all 16,180 full-year members of a large HMO who had diagnosed type 2 DM and (2) a like number of control members matched on year of birth and gender. We compared the distribution of costs (inpatient, outpatient, and pharmacy) among those with and without CVD and DM. We then profiled costs for diabetic subjects with and without CVD along the dimensions of age, gender, duration of diabetes and glycemic control.

Results: CVD was 76% more prevalent in persons with diabetes than in the control group (28.7% vs. 16.3%, $p < .001$), and those with DM were more likely to have multiple CVD conditions (14.2% vs. 6.7%, $p < .001$). Inpatient costs accounted for 31% of total costs in persons without CVD, but 51% of the total when CVD was present, regardless of DM status. Although costs were considerably higher across all strata when CVD was present, patterns of costs across dimensions differed. For example, costs among those with CVD peaked in the 55-64 age group and then declined with age. Among those without CVD, however, costs grew steadily with age. Differential cost profiles also emerged across duration of diabetes and glycemic control categories.

Conclusions: Although prior studies have established the excessive costs of CVD and DM, the current study provides important new information about the differential impact of CVD on medical care costs. CVD affects the distribution of cost components as well as producing cost profiles across patient characteristics that differ from those of patients without CVD.

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SOCIOECONOMIC DISPARITY IN CORONARY HEART DISEASE MORBIDITY AND MORTALITY AMONG DIABETIC PEOPLE IN FINLAND

E. Forssas, I. Keskimäki, S. Koskinen, and A. Reunanen. STAKES, Outcomes and Equity Research; National Public Health Institute, Department of Health and Disability. **Background and Aims:** Coronary heart disease (CHD) is an important complication of diabetes mellitus and the main reason for the increased mortality among diabetic people. The study analyses excess CHD morbidity and mortality and their patterning according to socioeconomic status (SES) in a nation-wide cohort of diabetic people.

Materials and Methods: Data on Finns aged 35-74 entitled to reimbursement for antidiabetic medicines (n=62503) and on non-diabetic referents (n=123709) came from the 1990 Social Insurance Register. The data were individually linked to the 1991-1996 Social Insurance Register on chronic diseases, and National Hospital Discharge and Causes of Death Registers. SES data were obtained from the population censuses.

Results: In 1996 the prevalence of persons entitled to reimbursement for CHD medicines was almost three times higher among diabetic women (8.3%) and over two times higher among diabetic men (12.3%) than among referents. Compared to 1990 figures the highest increase in prevalence was in female lower white-collar (9%) and in male blue-collar workers (11%) with diabetes. Overall CHD incidence and mortality rates decreased markedly in 1991-1996. From 1991-1993 to 1994-1996 CHD morbidity and mortality disparities increased in diabetic women according to disposable family income but remained stable according to social class and education. Among diabetic men no change in SES disparities occurred in the follow-up. In 1994-1996, the CHD incidence and mortality rate in the lowest income quintile related to the two highest quintiles were 1.63 (95%-CI:1.37-1.94) and 1.74 (1.47-2.07) for diabetic women, and 1.34 (1.15-1.55) and 1.48 (1.30-1.68) for diabetic men. In general, SES differences in CHD incidence and mortality were larger among non-diabetic than diabetic people.

Conclusions: The prevalence of CHD increased in diabetic and non-diabetic people, although CHD incidence and mortality decreased. Overall CHD morbidity and mortality were markedly higher but their SES differences smaller among the diabetic than non-diabetic people. SES disparities among diabetic people remained stable, except in the lowest income quintile, where women's risk for CHD morbidity and mortality increased.

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THE RELATIONSHIP BETWEEN CARDIOVASCULAR RISK AND HOSPITAL BED UTILISATION IN PEOPLE WITH TYPE 2 DIABETES

C.J. Currie¹, P. McEwan², P. Hopkinson³, and J.R. Peters⁴. ¹University Hospital of Wales, Cardiff, UK; ²Cardiff University, UK; ³Glaxo Wellcome, London, UK **Background and Aims:** The purpose of this study was to classify patients with Type 2 diabetes (T2D) by their predicted CHD risk using the Cardiff Risk Function, and to estimate the associated UK hospital utilisation of the various risk groups, as a proxy for financial treatment costs. **Materials and Methods:** Using the Cardiff Risk Function and data abstracted from the Cardiff Diabetes Database, patients with T2D were classified into three groups based on their four-year predicted probability (pp) of a CHD event. Low risk was defined as a four-year pp≤0.17, medium risk as 0.17<pp≤0.25 and high risk was defined as pp>0.25. The mean number of days spent in hospital from 1996 to 1999 inclusive was used as a proxy for hospital costs for all admissions for macrovascular disease events. **Results:** Results are reported using the mean number of bed days by category, cross-tabulated by risk and gender. Of the 2718 patients studied, 56% had at least one hospital admission during the period. There were 1480 males (mean four-year pp=0.23 [SD=0.10]) and 1238 females (mean four-year pp=0.19 [SD=0.09]). Mean bed days per risk category are tabulated below. **Conclusion:** There is a defined utilisation - thus cost - gradient associated with categories of cardiovascular risk in patients with T2D. This study demonstrates that significant cost savings could be achieved by reducing the prevalence and severity of CHD complications associated with T2D.

	Low risk, mean (SD)	Medium risk, mean (SD)	High risk, mean (SD)	All, mean (SD)
Male	8.7 (27.1)	14.2 (29.4)	22.7 (45.4)	16.1 (36.8)
Female	12.4 (29.9)	17.3 (28.7)	28.7 (45.8)	18.1 (36.4)
All	10.6 (28.7)	16.0 (31.9)	24.7 (45.6)	17.0 (36.6)

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THE VALIDITY OF THE UKPDS RISK EQUATION FOR PREDICTING THE LIKELIHOOD OF CARDIOVASCULAR HEART DISEASE EVENTS IN TYPE 2 DIABETES

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Background and Aims: The Framingham risk function has been demonstrated to be unreliable for predicting the likelihood of CHD events in Type 2 diabetes (T2D). Data from the UKPDS have been used to derive a risk equation specifically for people with T2D. The purpose of this study was to test the hypothesis that the UKPDS risk equation would better predict the likelihood of CHD events in a T2D population than would the Framingham equation.

Materials and Methods: Data were abstracted from the Cardiff Diabetes Database that respected the age restrictions for the respective risk functions (n=507). The register had no exclusion criteria. Of 101 CHD events, 35% were in female subjects. The predicted probabilities of a CHD event were derived and evaluated using receiver operator characteristic (ROC) curves. The predictive value of a positive test was determined using the upper quartile of risk for each equation to compensate for differences in scale.

Results: The area under the ROC curve was 0.64 for the original Framingham equation, 0.65 for an optimised Framingham equation, and 0.62 for the UKPDS equation. An equation derived on these data returned an ROC value of 0.68. In the same respective order, the predictive value of a positive test was as follows: 0.29, 0.26, 0.26, and 0.32. The null hypothesis was therefore accepted.

Conclusions: It was concluded that the UKPDS risk function did not offer any notable improvement on the Framingham risk equation in predicting the likelihood of CHD events in T2D. due to the age restrictions for the UKPDS risk function, 56% of subjects with T2D were excluded from use in the UKPDS equation. Twenty-eight percent of patients in the Framingham equation were excluded. All three equations had limitations and require identification and incorporation of additional risk components to improve their predictive ability.

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TYPE 2 DIABETES IMPACT ON QUALITY-ADJUSTED LIFE EXPECTANCY

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Background and Aims: To estimate the effect of Type 2 diabetes in terms of quality-adjusted life-years (QALYs) lost. **Materials and Methods:** EuroQol scores obtained in the Cost of Diabetes in Europe-Type 2 (CODE-2) study were applied to estimates of life expectancy and the prevalence of complications, produced by an established computer model of Type 2 diabetes, for a typical cohort of newly diagnosed cases followed until death. These were compared with results generated for a similar non-diabetic cohort. Two aspects of uncertainty were addressed by sensitivity analysis: the mean EuroQol score appropriate to the non-diabetic cohort, and the effect of multiple complications on the combined EuroQol score. Two methods of calculating scores for multiple complications were used: using the worst score for the complications present, and multiplying together the incremental effects of each complication. **Results:** Varying the EuroQol score for non-diabetic patients had only limited effect on the size of the effects, and did not alter the relative importance of complications. **Conclusions:** When expressed in QALYs, the effect on patients of Type 2 diabetes is very large compared with non-diabetic patients. The importance of multiple complications reinforces the need for early intervention.

Basis for estimating EuroQol score for multiple complications (per patient)	Multiplying incremental effects	Using worst score
Expected QALYs (non-diabetic patients)	23.0	23.0
QALYs lost to Type 2 diabetes		
Reduction in life expectancy	-3.5	-3.5
Living with diabetes (no complications)	-0.5	-0.5
Living with diabetes (single complication)	-1.7	-1.7
Living with diabetes (2+ complications)	-4.8	-3.2
Total	-10.5 (-45.5%)	-8.9 (-38.6%)
Expected QALYs (Type 2 diabetic patients)	12.5	14.1

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Exercise

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EVIDENCE FOR SPATIAL HETEROGENEITY IN INSULIN- AND EXERCISE INDUCED INCREASES IN GLUCOSE UPTAKE

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Background: It is unknown whether resistance to insulin- or exercise-stimulated glucose uptake reflects a spatially uniform or nonuniform decrease in glucose uptake within skeletal muscle. **Materials and Methods:** We compared the distributions of muscle glucose uptake and blood flow in eight patients with type 1 diabetes (age 24 ± 1 years, BMI 22.0 ± 0.8 kg/m²) and seven age- and weight-matched normal subjects using positron emission tomography, [¹⁸F]-fluoro-deoxy-glucose and [¹⁵O]-water. Both groups were studied during euglycemic hyperinsulinemia and one-legged exercise. Heterogeneity was evaluated by calculating relative dispersion (standard deviation divided by mean $\times 100$ %) of glucose uptake (RD_g) and flow (RD_f) in all pixels within a region of interest in femoral muscle. **Results:** The exercise-induced increment in glucose uptake but not in blood flow was significantly lower in the type 1 diabetic patients than in the normal subjects (94 ± 21 vs 186 ± 29 μ mol/kg·min). RD_g, but not RD_f, was increased in the insulin resistant type 1 diabetic patients both at rest (RD_g 31 ± 1 vs 25 ± 2 %, patients with type 1 diabetes vs normal subjects, $p < 0.05$) and during exercise compared to normal subjects (27 ± 1 vs 21 ± 2 %, respectively, $p < 0.05$). Exercise increased both glucose uptake and blood flow several-fold and significantly decreased both RD_g and RD_f. RD_g was inversely associated with total glucose uptake ($r = -0.54$, $p < 0.001$, pooled data) and was highest in the most insulin-resistant patients. **Conclusions:** We conclude that both glucose uptake and blood flow are characterized by heterogeneity in human skeletal muscle, which magnitude is inversely proportional to respective mean values. This implies that an increase in glucose uptake in human skeletal muscle is not a phenomenon, where each unit increases its glucose uptake by a fixed amount but rather a spatially heterogeneous process.

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EFFECT OF EXERCISE TRAINING ON TUMOR NECROSIS FACTOR- α SYSTEM, INSULIN SENSITIVITY AND PLASMA LEPTIN IN OBESSE WOMEN.

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Background and aims: Tumor necrosis factor- α (TNF α) may play an important role in the pathogenesis of insulin resistance and type 2 diabetes. Plasma levels of the soluble fractions of TNF α receptors, especially sTNFR2, are good indicators of TNF α system activation in obesity. The aim of the present study was to assess the effect of exercise training on TNF α system and to evaluate the relationship with the changes in insulin sensitivity and plasma leptin. **Materials and methods:** Sixteen overweight and obese women (BMI > 27.8 kg/m²): 8 with normal (NGT) and 8 with impaired glucose tolerance (IGT), participated in the exercise training program, which lasted for 12 weeks and included exercise performed on bicycle ergometer at an individual intensity of 70% maximal heart rate, 30 min 5 days a week. Anthropometrical measurements and blood biochemical analyses were performed, and plasma TNF α , sTNFR1, sTNFR2 and leptin levels were assessed. Insulin sensitivity was evaluated using the hyperinsulinemic euglycemic clamp technique (insulin infusion: 50 mU \times kg⁻¹ \times hour⁻¹) and normalized for fat-free mass. **Results:** At baseline, despite similar anthropometrical parameters, IGT subjects were markedly more insulin resistant and had higher TNF α and sTNFR2 concentrations. Exercise training increased insulin sensitivity and decreased TNF α and sTNFR2 levels, while sTNFR1 remained unchanged. Decrease in sTNFR2 was significantly related to the increase in insulin sensitivity ($r = -0.70$; $p < 0.005$), and to the decrease in plasma leptin ($r = -0.51$; $p < 0.05$). The relationship between changes in sTNFR2 and insulin sensitivity remained significant after adjustment for the concurrent changes in BMI, WHR, percent of body fat, plasma glucose and free fatty acids. **Conclusions:** Regular physical exercise decreases TNF α system activity and that decrease may be responsible for the concurrent increase in insulin sensitivity.

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EFFECT OF EXERCISE ON BODY FAT MASS, SOLUBLE FRACTION OF TNF RECEPTORS, LEPTIN AND INSULIN SENSITIVITY

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Aims: Obesity and type 2 diabetes are associated with increased resistance to insulin. Tumor necrosis factor- α (TNF- α) was reported to inhibit insulin action and to play a role in insulin resistance. The soluble fraction of the TNF receptors 1 and 2 (sTNFR1 and sTNFR2) are thought to reflect the degree of activation of the TNF system. We studied the effect of exercise training on body fat mass, the TNF system and insulin sensitivity using euglycemic hyperinsulinemic clamp combined with an oral glucose. **Methods:** Subjects were divided into two groups: Fifteen patients were managed by diet alone (Diet group), and twenty-one patients were managed by diet and exercise (Exercise group). We calculated insulin-mediated glucose uptake by the liver and peripheral tissues before and after treatment. **Results:** Body weight, BMI, total grams of fat and lean tissue mass, FPG, IRI and serum total cholesterol were decreased significantly in both groups after treatment. While the %fat of body composition decreased significantly 33.6 ± 3.4 % to 31.3 ± 3.6 (Mean \pm SE) in the Exercise group, but remained unchanged in the Diet group after treatment. The glucose infusion rate increased 4.1 ± 0.3 mg/kg/min to 4.8 ± 0.3 in the Exercise group, but remained unchanged in the Diet group. Hepatic glucose uptake and TNF- α remained unchanged in both groups. Plasma concentrations of sTNFR1 and leptin decreased significantly in both groups. **Conclusion:** Weight loss induced the decrease of sTNFR1 and leptin. Furthermore, exercise training decreased % Fat of body composition and improved insulin sensitivity in peripheral tissue.

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1 HOUR A WEEK OF EXERCISE IS ENOUGH: A STUDY OF A 3 AND 6 MONTH EXERCISE PROGRAM IN PATIENTS WITH DIABETES.

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Background and Aims: Reduced physical activity has been identified as a major risk factor for Coronary Heart Disease (CHD), with its contribution being as great as smoking and hyperlipidaemia. The exact intensity and frequency of activity needed to offset this risk is not known. In this study we aimed to assess whether a once weekly supervised exercise class would be sufficient to improve cardiac risk factors in patients with diabetes and if so at what time interval improvements would be seen.

Materials and Methods: Patients who expressed an interest in attending a supervised exercise program were referred from their diabetic clinics. No exclusion criteria were applied and places on the program were offered on a first come first served basis. 20 people at time were enrolled into the program which consisted of an hour long exercise class followed by half an hour of education each week. The first program ran for 6 months and the subsequent three programs for 3 months. Prior to and on completion of the exercise program patients attend for measurement of height, weight, waist circumference, HbA1c and cholesterol concentrations and completed a well being questionnaire.

Results: 56 patients (40 F) participated in the 3 month program. Mean age was 60 (34-73) and 52 had type 2 and 4 type 1 diabetes. This exercise program significantly improved well being (22 ± 1 Vs 27 ± 1 , $p < 0.001$) and waist circumference (42.9 ± 0.97 Vs 41.8 ± 0.96 , $p < 0.00001$). Body mass index (BMI) showed a trend towards improving (33.9 ± 0.96 Vs 33.4 ± 0.91 , $p = 0.10$), but exercise had no effect on HbA1c (8.4 ± 0.2 Vs 8.3 ± 0.2 , $p = 0.35$) or cholesterol (5.4 ± 0.16 Vs 5.1 ± 0.13 , $p = 0.18$). 18 patients (13 F) took part in the 6 months exercise program. Mean age was 62 (45-77) and 13 had type 2 and 5 had type 1 diabetes. This exercise program significantly improved well being (43 ± 3 Vs 54 ± 3 , $p < 0.01$), waist circumference (40.7 ± 1.6 Vs 38.9 ± 1.6 , $p < 0.001$), body mass index (31.3 ± 1.5 Vs 30.8 ± 1.6 , $p < 0.05$), cholesterol (5.5 ± 0.3 Vs 5.2 ± 0.23 , $p = 0.05$) and HbA1c (8.9 ± 0.3 Vs 8.4 ± 0.3 , $p < 0.01$). Triglycerides also showed a trend towards improving (2.28 ± 0.26 Vs 1.98 ± 0.23 , $p = 0.13$). Gender or Type of diabetes was not predictive of the response to exercise but those who started with the lowest well being score improved the most ($r = 0.94$, $p < 0.001$).

Conclusions: 1 hour a week of supervised exercise is enough to significantly improve many risk factors for CHD, provided exercise is continued for at least 6 months. What percentage of individuals persist with this exercise pattern once they are left on their own still remains to be determined.

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PHYSICAL ACTIVITY BEHAVIOUR AND CORRELATES OF LOW PHYSICAL ACTIVITY LEVELS IN PEOPLE WITH TYPE 2 DIABETES.

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Background and Aims: Physical activity is important for Type 2 diabetes management. This study evaluates physical activity and related factors in people with Type 2 diabetes. **Materials and Methods:** 36 people with Type 2 diabetes (21M 15F, age 56.9±7.4years) completed physical, metabolic and quality of life assessments. Peak VO₂ (ml/kg/min) was assessed and physical activity was measured by 7-day recall, stage of exercise behaviour and an accelerometer. **Results:** Participants achieved on average 102±152 minutes of moderate activity/wk and a peak VO₂ of 20.6 ± 6.1. Participants in preparation stage of exercise behaviour recorded higher moderate intensity activity/wk (p=0.02) and activity counts/wk (p=0.00) than participants in contemplation stage, no difference was shown in peak VO₂. The strongest positive associations were minutes of moderate activity/wk with positive well-being, total general well-being and physical functioning. The strongest negative associations were total activity counts/wk and peak VO₂ with BMI(see table). **Conclusions:** Participants were inactive with low cardio-respiratory fitness. Findings suggest BMI and quality of life are important determinants of physical activity in people with Type 2 diabetes.

Table – Variables associated with physical activity levels. *p<0.05; **p<0.001

Variable	Moderate activity/wk	Activity counts/wk	Peak VO ₂
BMI	-0.3*	-0.5*	-0.5**
Depression	-0.4*	-0.2	-0.06
Anxiety	-0.3*	-0.2	-0.08
Energy	0.3	0.4*	0.2
Positive well-being	0.5*	-0.002	-0.1
Total general well-being	0.5*	0.3	0.06
Physical functioning	0.5*	0.2	0.4*
Energy/vitality	0.4*	0.1	0.3*
Less pain	0.3*	0.2	0.1

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Catch-up growth and glucose tolerance in children

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Background and Aims: Childhood catch-up growth (CUG) occurs between birth and 2 years of age and is a response to poor fetal growth. Studies have shown that rapid weight gain between birth and 7 years in low birth weight children has a negative effect on glucose tolerance. Therefore, the aim of this study was to determine if CUG affects childhood glucose tolerance.

Materials and Methods: Oral glucose tolerance tests were performed on 7-year-old children from the Birth to Ten (B10) study who displayed catch-up growth (n=23), normal growth (NG; n=29) and catch-down growth (CDG; n=29). Blood samples were taken at 0, 30 and 120 minutes and analysed for insulin, glucose, proinsulin and des-31, 32 proinsulin. The B10 study is a longitudinal investigation of childhood health and welfare in 4000 children born in the Soweto-Johannesburg conurbation. The cohort used in our study was selected randomly from African children for whom birth weight and weight and height at 2, 4, 5 and 7 years of age were available. Head circumference and skinfold thickness were available at 2, 4 and 5 years of age.

Results: (mean±SD): Birth weight was lower in the CUG than CDG group (2.9±0.5 vs 3.5±0.4 kg; p<0.001) whilst weight at 2 was higher (13.5±1.9 vs 10.6±1.3 kg; p<0.001). Weight velocity between 1 and 2 years of age was higher in the CUG than CDG group (3.1±1.2 vs 1.4±1.0 kg/year; p<0.001) whilst weight velocity between 2 and 4 years was lower (1.2±1.3 vs 2.3±0.8 kg/year; p<0.001). No differences in height or head circumference were noted at any age however arm circumference (17.0±1.1 vs 15.6±1.2mm; p<0.01), tricep (6.6±2.4 vs 5.2±1.5mm; p<0.05) and subscapular (6.9±1.8 vs 5.8±1.0mm;p<0.05) skinfold thickness' were significantly higher in the CUG than CDG children at 2 years. The NG children had values intermediate between those of the CDG and CUG groups for all these variables. Weight at 7 was the same in all 3 groups. The fasting, 30 and 120 minute insulin, proinsulin, des-31, 32 proinsulin and glucose levels were not different between the groups.

Conclusions: CUG and CDG are mainly due to relative changes in soft tissue mass. CUG is followed by a period of slower and CDG by a period of faster growth after the age of 2 resulting in similar weights for the 3 groups by the age of 7. Neither CUG or CDG affects glucose tolerance. Therefore, CUG on its own does not have a negative effect on childhood glucose tolerance. This suggests that the poorer glucose tolerance observed in the low birth weight children who display relatively high childhood weight gain may be the result of a sustained period of rapid growth which exceeds that of CUG.

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Insulin Resistance and its Metabolic Impact in Five-Year-Old Children

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Background and Aims: Insulin resistance (IR) is believed to underlie type 2 diabetes and the metabolic syndrome, but it is unclear how early in life IR is acquired nor how strong its impact. The hypothesis is that IR, and the metabolic disturbances it causes, are acquired (rather than programmed) early in childhood and are, as such, preventable.

Materials and Methods: EarlyBird is a prospective cohort study monitoring 300 healthy children from school entry at 4/5 years through childhood. The cohort was reviewed at six-month intervals for anthropometry and measures of IR by homeostasis model assessment (HOMA), glucose, blood pressure and lipids. Physical activity (CSA accelerometer) and resting metabolic rate (indirect calorimetry) are added annually. Baseline data from the first 155 children (mean 4.8 y) and their parents are presented here.

Results: The girls (G) were of the same body weight (mean 19.5 kg), but of higher BMI with lower waist/hip ratios than the boys (B), and were significantly more insulin resistant (p<0.001). There was no correlation between IR at 5y and birthweight in either sex. There were, however, strong correlations between IR and current weight in mothers (r=0.66) and daughters (r=0.41), less so in fathers (r=0.52) and sons (r=0.11). Fasting insulin levels correlated strongly with IR over the range of IR in the children (r=0.98, p<0.001) but decreasingly so in those adults with higher IR. Glucose (B r=0.33; G r=0.28) and LDLChol (G r=0.25) were significantly correlated with IR at 5y (p<0.05), and variably with current weight.

Conclusions: 1) Weight-related metabolic disturbances are already detectable at school entry. 2) Weight at birth does not predict IR in today's children, while current weight best predicts IR. 3) The relationships at 5y between IR and body size, and between IR and metabolic disturbance, were confined to girls. The reasons for sexual diversity at such a young age are not clear, although differences in physical activity (also measured by the EarlyBird Study) may be a factor. 4) IR, and the metabolic disturbances which result, are potentially preventable by avoidance of early weight gain.

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The National Paediatric Diabetes Audit

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Background and Aims: The National Paediatric Diabetes Audit was initiated in July 1999 by Diabetes UK with the overall aim of facilitating, within the next 5 years, a national audit mechanism to develop a cycle of continuous quality improvement in paediatric diabetes care throughout the UK. The project aims to compare care provision for children with diabetes in different areas of the UK, to monitor Declaration of Kos targets, to measure intermediate outcomes relevant to children (HbA1c, hypos, hospital admissions) and to provide a resource for investigations into effective audit methodologies for diabetes in children & adolescents.

Materials and Methods: Anonymous demographic data and simple intermediate outcomes in the year 2000 for children aged 0-16 were collected and aggregated centrally. Data was collected from 49 centres within the UK, creating a cohort of 5023 patients. Informed written consent was gained from patients. Data fields were date, NHS number, postcode at diagnosis, ethnicity, sex, truncated date of birth, truncated date of diagnosis, type of diabetes, current postcode, death during the year, number of admissions with diabetic ketoacidosis (DKA) during the audit year, number of severe hypos during the audit year, the latest HbA1c result and the DCCT status of the testing laboratory. Postcodes were converted to Carstairs deprivation quintiles. Data was analysed using SPSS.

Results: The mean age is 11.4±3.5 years (n=5023), and the mean duration of diabetes is 4.5±3.4 years (n=4919). The mean age at diagnosis is 7.4±4.0 years (n=4984). 59.8% have ethnicity recorded (n=3000). Of these, 90.2% are Caucasian (n=2705). 46.9% are female (n=2354). 83.5% have type of diabetes recorded (n=4447). Of these, 98.6% have Type 1 diabetes (n=4386). 55.7% have a DCCT-standardised HbA1c result (n=2797). 55.6% have a record of whether they have been admitted for DKA (n=2795) and 51.9% have a record of whether they have experienced a severe hypo (n=2605). No patients have died. Significant associations with increasing HbA1c level are found for centre (p<0.001), ethnicity (any ethnic minority vs. white, p<0.0001), deprivation (the 2 most deprived quintiles vs. the 2 least deprived quintiles, p=0.002), increasing age (15-16 years vs. 0-4 years, p<0.0001) and duration of diabetes (10 years vs. 1 year duration, p<0.0001). Deprivation significantly affects the number of admissions for DKA (the 2 most deprived quintiles vs. the 2 least deprived quintiles, p=0.001).

Conclusions: Poorer outcomes for children with diabetes are associated with increasing deprivation, increasing age, increasing duration of diabetes, and non-white ethnicity. These results will be fed back to participating centres, allowing them to focus the care they provide.

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INSULIN AUTOANTIBODIES AS AN AGE DEPENDENT RISK MARKER FOR TYPE 1 DIABETES IN CHILDREN OF DIFFERENT POPULATIONS

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Background and Aims: Insulin is only one specific autoantigen for B cells. So, its prediction power for type 1 diabetes seems to be higher than other markers are. Aim of this study was to compare insulin autoantibodies (IAA) appearance in newly diagnosed diabetic children from populations which are characterised by different incidence rate for type 1 diabetes in 0-15 yr. old children i.e. Colorado 18-20/100,000 yr. and Wielkopolska 8.6/100,000/yr.

Materials and Methods: newly diagnosed diabetic children (0-15 years old) from Wielkopolska (Poland) and Colorado (USA) were divided into subgroups according to sex and age. Sera from patients, as well from their siblings and parents, were investigated for IAA presence. IAA were measured by radioimmunoprecipitation method using I125 labelled insulin and results (expressed in arbitrary units - AU) were positive when exceeded 42 AU/ml. Another autoantibodies (GADab, ICA512) were estimated too.

Results: IAA as only one autoantibody (10.3% vs. 5.3%) or even coexisting with others (65.4% vs. 51.11%) were found more frequently in Colorado than in Wielkopolska children (x²-test), however, their appearance was dominating in the youngest subgroups in both populations: in Colorado 0-4 years old: 90.9%, 5-9yr.: 76.7%, 10-15yr.: 53.8% and in Wielkopolska 100%, 60%, 41.8% respectively. Boys seemed to be more prone than girls to reveal IAA in both cohorts, however without statistic significance. Additionally, IAA serum level (AU mean ± SD) was highest in the youngest children in Colorado (0-4 years old: 1413±1281, 5-9yr.: 693±732, 10-15yr.: 343±563), when in children from Wielkopolska its concentrations were highest in the oldest subgroup (0-4 yr.: 288±193, 5-9yr.: 618±731, 10-15yr.: 1105±1455) if evaluated by Mann-Whitney statistics. When IAA were found together with other autoantibodies, their age dependent profile overcame characteristics specific for GADab and ICA512 distribution.

Conclusions: IAA are the most predictive risk marker for type 1 diabetes in the youngest children. Their appearance is in relation to type 1 diabetes incidence rate in different populations.

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CLINICAL AND SUBCLINICAL ORGAN-SPECIFIC AUTOIMMUNE MANIFESTATIONS IN TYPE 1 DIABETIC CHILDRENA. Körner, A. Arató and L. Madácsy, 1st Dept. Pediatrics, Semmelweis University, Budapest, Hungary

Background: Autoimmune disorders (AD) are known to be clustered in the same person. The aim of the study was to evaluate the prevalence of other AD in our patients with type 1 DM. **Material and Methods:** Four hundred children (age: 1.1-19.25 years) with type 1 DM have been investigated. Diagnosis of different AD was made either by clinical symptoms or by specific serological screening. Screening for celiac disease (CD) was performed in 194 diabetic children by anti-endomysium antibody (AEA) test. In subjects, tested positive for AEA, diagnosis of CD was confirmed by jejunal biopsy. Screening for thyroid disease (TD) was performed by anti-thyroglobulin and anti-thyroid peroxidase antibodies in 90 diabetic patients parallel to the determination of serum thyroid hormone and thyroid stimulating hormone levels. **Results:** Overall, 42 (21.6 %) AEA positive subjects were detected. Diagnosis of CD was confirmed by jejunal biopsy in 31 children (16 %). High titers of at least one thyroid related auto antibody were present in 12 out of 90 diabetic children (13.3 %). Among those, tested positive for anti-thyroid antibodies, hyperthyroidism was diagnosed in 2 children (2 %) and hypothyroidism was found in 6 patients (6.7 %). In the whole diabetic population 29 children (7.3 %) had vitiligo, and juvenile rheumatoid arthritis was diagnosed in 1 child. Co-occurrence of type 1 DM with other AD was observed in 62 children (15.5 %). Fifty five diabetic patients (13.8 %) were suffering from 1; 6 patients (1.5 %) in 2 additional AD. One diabetic patient had type 3 polyglandular syndrome. Age at onset of diabetes appeared to be significantly (p<0.05) lower in patients with multiple AD than in children with DM alone. **Conclusions:** In diabetic children, the most prevalent autoimmune manifestations are celiac disease and thyroid disorders. Both diseases can be initially clinically silent, detected only by specific screening tests. Early onset type 1 DM confers an increased risk for multiple autoimmune disorders.

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CHARACTERIZATION OF 48 PAEDIATRIC PATIENTS WITH DOWN SYNDROME AND DIABETES MELLITUS.

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Background and Aims: While most paediatric patients with diabetes are type-1, rare forms of the disease do exist in childhood and adolescence. An increased incidence of diabetes is well recognised in patients with Down syndrome (trisomia 21), currently classified as type-3 diabetes, other genetic syndromes associated with diabetes. While several case-reports illustrate this association, few reports on large groups of patients with Down syndrome and diabetes mellitus do exist so far.

Materials and Methods: In Germany, most paediatric diabetes centres use a common structured prospective documentation system, DPV. Anonymized data are available for centralized descriptive analyses. By March of 2001, this database consisted of 15 126 diabetes patients younger than 20 years of age, which were treated at 117 paediatric centres in Germany and Austria.

Results: 48 patients (20 boys, 28 girls) were diagnosed with Down syndrome. These patients were compared to 14809 patients with type-1 diabetes (7713 boys, 7096 girls). The mean age at diagnosis in patients with Down-syndrome and diabetes was 7.25 years compared to 7.98 years in paediatric patients with type-1 diabetes (n.s.). At the most recent exam, age averaged 14.4 years (13.0 in type 1 diabetes). Height-SDS was -2.47 ± 1.09 for DM-T21 (+0.30±1.16 for T1-DM). BMI > the 90th percentile was present in 20.8 % of DM-T21 (17.9 % in T1-DM). Insulin dose averaged 0.86 U/kg and day in DM-T21 (0.83 U/kg in T1-DM). On average, patients with T21-DM received 3 injections per day compared to 3.5 injections in T1-DM (p<0.0001). Metabolic control tended to be better in DM-T21 (mean HbA1c: 7.63±1.37 %) compared to T1-DM (8.22±1.93 %; p=0.06).

Conclusions: In a large paediatric sample, diabetes in Down syndrome follows type-1, type-2 and CF-related diabetes in prevalence. Patients with Down syndrome are significantly smaller and tend to be more overweight. While more patients are on conventional therapy, insulin doses and metabolic control are similar.

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Infantile Type I Diabetes Mellitus and Acute Liver Failure. A New Mitochondrial Depletion Syndrome?

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Background and Aims: Insulin-requiring diabetes presenting in the first few months of life is rare, with an incidence in the first 3 months of 1 in 450,000. At least 50% of these children have only transient insulin requirements. Of those with permanent diabetes, only three sets of siblings have been described in the literature.

Materials and Methods: We report a consanguineous pedigree with four children: non-identical twins in one family and a sib pair in the second.

Results: All developed insulin-requiring diabetes within the first four months of life. Three of the four affected individuals developed acute liver failure during intercurrent viral illnesses and died. Post-mortem histology revealed acute fatty degeneration of the liver in all three children, as well as a reduction in the size of the Islet cells within the pancreas. The fourth child now aged 10 is still insulin dependent, has growth failure, chronic renal impairment, and liver disease which has been shown to be exacerbated by intercurrent infections. We found no reports of Type I diabetes and coexisting liver disease in the literature.

Extensive investigation of the affected sib pair has shown a Mitochondrial Depletion Syndrome. Mitochondrial DNA levels were reduced to 16% of normal values, in muscle tissue, obtained from the sibling of the surviving child.

Conclusions: This is the first report of permanent early onset Type I diabetes and liver dysfunction in association with a Mitochondrial Depletion Syndrome

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PREPRANDIAL VERSUS POSTPRANDIAL INSULIN ASPART TREATMENT IN TYPE 1 DIABETIC CHILDREN AND ADOLESCENTS

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Background and Aims: While insulin is usually injected preprandially (PREP) to obtain adequate postprandial glycemic control, postprandial (POP) injection would offer the advantage of meal size adjusted dosage. This trial compared the glycemic control of PREP vs. POP injection of insulin aspart (IAsp) in children and adolescents with type 1 diabetes.

Materials and Methods: 76 children (6-12 years) and adolescents (13-17 years) were randomized to PREP and POP treatment with IAsp as part of a basal-bolus regimen in a multicentre study (9 pediatric centers in Austria, Germany and Sweden) with an open labeled, two period cross-over design (six week periods). PREP being immediately before meal start, and POP after the meal (max. 30 min. after meal start). Baseline characteristics were: 55% <13 years; 49% boys; diabetes duration: 3.7 (1.0-9.4) years; baseline HbA1c: 7.6 (4.7-11.4)%. Standard dose-adjustment algorithms were used for dose optimization based on self-measured blood glucose levels.

Results: Glycemic control for POP treatment was non-inferior to PREP treatment as assessed by fructosamine (0 vs. 6 weeks: PREP: 367±74 vs. 378±90; POP 383±83 vs. 385±77 μmol/l; p=0.24) and HbA1c (PREP: 7.9±1.5 vs. 8.0±1.5%; POP 8.0±1.4 vs. 8.3±1.5%, p=0.14). Also, the average blood glucose concentration on profile day (PREP vs. POP: 8.5±0.3 vs. 9.2±0.4 mmol/l; p=0.08), and the average prandial blood glucose increment (-0.28±0.23 vs. -0.10±0.38; p=0.74) was comparable. Three severe hypoglycemic episodes occurred (PREP: n=2; POP: n=1). A total of 1007 hypoglycemic episodes (blood glucose <3.9 mmol/l) were recorded during the study with no difference between treatment regimens (relative risk PREP to POP 1.1 (0.9-1.3), p=0.31). No significant differences were found between children and adolescents in any of the parameters above. No safety concerns were raised during the trial (frequency of drug related adverse events or changes in hematology/biochemistry). Treatment satisfaction was equally high in patients and parents with both regimen.

Conclusions: Although preprandial administration of IAsp generally is preferable, our study shows that in children and adolescents postprandial administration of IAsp is a safe and effective alternative.

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IMPAIRED GLUCOSE TOLERANCE, INSULIN RESISTANCE AND INFLAMMATORY MARKERS IN OBESE CHILDREN.

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Background: Type 2 diabetes is a growing problem in childhood and seems to be related to an increasing rate of obesity. **Aims:** In a cohort of obese children of Caucasian origin we have a) studied the prevalence of IGT, IFG and diabetes, b) determined the contribution of insulin resistance/secretion to glucose tolerance and c) examined inflammatory markers related to risk of diabetes and cardiovascular disease. **Subjects and Methods:** 574 obese children (282 males, 292 females), mean age 14 yr (range 6.3-17), mean relative body weight (RBW) 183±29% were studied between 1994 and 2000. A 75g OGTT was performed and samples for estimation of blood glucose (BG), insulin, lipid profile, fibrinogen (Fg) and C reactive protein (CRP) were taken. Insulin sensitivity was calculated by HOMA-IR and β cell function by insulinogenic index: $\text{ins}_{30'} - \text{ins}_{0'}/\text{glu}_{30'} - \text{glu}_{0'}$ ($\Delta\text{I30}/\Delta\text{G30}$). **Results:** None of the children had diabetes, 24 (4.2%) had IGT, 2 of whom also had IFG. Compared to NGT subjects, IGT subjects showed female preponderance (1.7:1), were slightly older (15 ± 2 vs 14 ± 3 yr, $p < 0.05$), had a stronger family history of type 2 diabetes (70 vs 50%, NS) and similar RBW. Tanner II-IV stages were 21% in IGT and 27% in NGT(NS). In a multivariate regression analysis, HOMA-IR was positively and $\Delta\text{I30}/\Delta\text{G30}$ was negatively and independently correlated with the 2hBG ($p < 0.0001$ for both). Fasting lipids, Fg and CRP were not correlated with 2hBG, but total triglycerides (TG) and Fg showed, in a regression model, a significant correlation with HOMA-IR ($p < 0.0001$ and $p = 0.042$ respectively). There was no correlation between these indices of inflammation and $\Delta\text{I30}/\Delta\text{G30}$. TG and Fg increased with pubertal development and with increasing RBW. **Conclusions:** In obese children the prevalence of IGT is high and appears to be dependent on derangements of both insulin sensitivity and insulin secretion. Levels of inflammatory proteins and triglycerides are not directly related to glucose intolerance, but TG and Fg are associated with the degree of insulin sensitivity. This suggests an aggregation and possible pathogenic sequence of risk factors for both diabetes and cardiovascular disease in obese children.

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Changing phenotype: Are more youngsters with IDDM obese at onset?

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It is generally believed that children diagnosed with insulin-dependent diabetes mellitus (IDDM) are not obese at onset. In order to evaluate if there has been a change in this assumption, we compared the prevalence of obesity (PO) (defined as BMI > 85th percentile) at onset in all Blacks < 20 years of age matched with Whites by age and year of diagnosis, diagnosed during two different periods: 1979-1989 (Period I) and 1990-1998 (Period II) and its relationship with demographic and autoimmune characteristics. There were 71 children diagnosed during Period I and 115 diagnosed during Period II.

The prevalence of obesity increased from 12.7% (Period I) to 36.5% (Period II) ($p = 0.0004$). The same increase was seen in both males and females. The prevalence of obesity in Whites increased from 2.8 (I) to 16.4% (II) ($p = 0.04$) and in Blacks from 22 (I) to 55% (II) ($p = 0.001$). In the younger age group (< 11 years), PO increased from 2.8% to 16.6% ($p = 0.06$) and in the older age group from 22% to 48% ($p = 0.009$). In children who were positive for at least one islet cell antibody (either ICA, GAD65 or IA-2) (86%), PO went from 8.2 to 29.9% ($p = 0.001$). However, PO in the group that had no autoantibodies (14%) was not significantly different between the two periods. In the multivariate logistic regression, period of diagnosis as well as race, age at onset and autoimmunity, were associated with obesity.

It is concluded that, at onset of IDDM, prevalence of obesity has more than doubled from the 1980's to the 1990's. This increase was seen in males and females, Whites and Blacks, adolescents and in children with evidence of autoimmunity. This leads to speculate that obesity may be an accelerating factor at onset of the disease and may be contributing to the increase in incidence seen in some populations.

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The role of self-efficacy in self-management of type 1 diabetes: Development and validation of the Confidence In Diabetes Self-care scale (CIDS) in Dutch and US patients.

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Background and Aims: Self-efficacy, the individual's perceived ability to perform diabetes self-care tasks, is predictive of actual self-care behaviour and of clinical outcomes in persons with diabetes. The objective of this study is to examine the psychometric properties of the Confidence In Diabetes Self-care (CIDS) scale, a newly developed instrument to assess self-efficacy in patients with type 1 diabetes in Dutch and US patients.

Materials and Methods: The CIDS, containing 20 items reflecting all aspects of self-care, was completed by 151 Dutch (NL) and 190 US patients. Other measures included the Problem Areas in Diabetes (PAID) and the Hypoglycemia Fear Survey (HFS). Different measures were used to assess self-esteem, self-care behaviour, anxiety and depression (all scores were transformed to a 0-100 scale). HbA1c was assessed.

Results: Samples show very similar characteristics (Age NL 43.2 sd 13.4; US 42.6 sd 13.1; Duration of DM NL 21.8 sd 13.0; US 22.2 sd 13.5; % Female NL 51.7; US 60.5; HbA1c NL 8.1 sd 1.3; US 8.3 sd 1.5; HFSworry NL 28.0 sd 16.8; US 30.7 sd 20.3; All NS; PAID NL 21.5 sd 16.0; US 36.0 sd 24.0, $p=0.000$) and CIDS scoring patterns (NL 83.0 sd 11.5; US 85.0 sd 12.5; NS). Scores indicate high levels of self-efficacy, with US men scoring significantly higher than US women (87.6 sd 11.2 vs. 83.4 sd 13.0 $p=0.02$). Internal consistency of the CIDS was high in both samples (Cronbach's α NL .85; US .89). Moderate correlations with other measures in the expected directions (PAID NL -.45**; US -.51**; HFSworry NL -.03; US -.34**) support construct validity while indicating that the CIDS reflects a unique construct ($*p<0.05$ ** $p<0.01$). Other constructs also showed moderate significant correlations with CIDS scores (Self-esteem NL .16*; US .36*; Self-care behaviour NL .47**; US .47**; Anxiety NL -.23**; US -.29**; Depression NL -.17*; US -.29**). HbA1c was significantly associated with CIDS scores in the US sample only (NL .09 NS; US -.36**). CIDS scores in a sample of type 1 patients in poor glycemic control taking part in a RCT aimed at improving HbA1c by means of Cognitive Behavioural Group Training were significantly lower (72.4 sd 12.8; $p=0.000$; $n=39$).

Conclusions: The Dutch and US version of the CIDS demonstrated high psychometric equivalency, allowing for cross-cultural comparison. Lower CIDS scores in a sample of patients in poor control support the usefulness of the CIDS as a screening tool to identify patients with suboptimal levels of self-efficacy.

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The quality of life of patients with type 1 and type 2 diabetes mellitus and impact on quality of diabetes care – JEVIN, a population-based survey

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Background: JEVIN (Jena's St. Vincent Trial) is a prospective, 10-years-follow-up, population-based survey of all insulin treated patients with type 1 and type 2 diabetes mellitus aged 16 to 60 years living in the city of Jena (100,000 inhabitants). **Methods:** In 1989/90 190 patients, in 1994/95 244 patients and in 1999/2000 362 patients (90% of the target population) were examined. Quality of life and treatment satisfaction were assessed using standardised questionnaires according to Bott et al. (subscales: social relations, leisure time flexibility, physical complaints, worries about future, diet restrictions, daily hassles, well-being, fear of hypoglycaemia). **Results: Type 1:** In 1999/2000 questionnaires of 102/133 patients (77%, age 44.4±12.3, diabetes duration 17.8±12.2 years, BMI 26.0±3.6 kg/m², HbA1c 7.89±1.53% [HPLC, Diamat®, normal range 4.5-6.3%]) were analysed. The diabetes duration was associated with social relations (R -square=0.057, $\beta=0.24$, $p=0.02$), symptoms of neuropathy (examined according to Young et al.) were associated with physical complaints (R -square=0.034, $\beta=0.21$, $p=0.042$) and both parameters were associated with worries about future (R -square=0.098, diabetes duration $\beta=0.23$, $p=0.031$, symptoms of neuropathy $\beta=0.28$, $p=0.009$). Patients with an HbA1c below 7% had a better well-being (80.7±19.3% [$n=47$] vs 70.7±22.4% [$n=55$], $p=0.018$). There were no associations between the number of insulin injections/day, the frequency of blood-glucose self-monitoring or intensified insulin therapy and quality of life. **Type 2:** Questionnaires of 192/229 patients (84%, age 56.8±7.6, diabetes duration 14.1±8.4 years, HbA1c 8.09±1.37%) were analysed. Patients with more than 2 insulin injections had a lower quality of life (58.0±20.9% [$n=94$] vs 65.5±19.2% [$n=98$], $p=0.011$) and poorer results in the subscales fear of hypoglycaemia (53.4±28.7% vs 64.0±25.4%, $p=0.008$), social relations (67.8±25.2% vs 76.2±21.5%, $p=0.014$) and worries about future (41.8±24.1% vs 49.7±26.0%, $p=0.031$). There was a positive correlation between the quality of life and the frequency of blood-glucose self-monitoring ($r=0.2$, $p=0.005$). **Conclusions:** In patients with type 1 diabetes intensified insulin therapy is not accompanied with lower quality of life, but with better quality of diabetes control. Patients with type 2 diabetes and more than 2 insulin injections per day had a lower quality of life. For these patients therapy should follow the most easy insulin regimen. Neuropathic complaints need a consequent treatment strategy.

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RANDOMISED TRIAL OF PROBLEM ORIENTED PSYCHOTHERAPY FOR TYPE 1 DIABETIC PATIENTS WITH COMPLICATIONS.

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Background and Aims: The presence of diabetic complications is significantly associated with psychosocial problems, lower quality of life and depression. Such patients often suffer from poor self-care resulting in sub-optimal metabolic control. Therefore, we have performed a randomised controlled trial on the effects of a problem-oriented, short-term psychotherapeutic intervention on self-defined psychosocial problems and metabolic control in Type 1 diabetic patients with microvascular complications. **Materials and Methods:** 46 Type 1 diabetic patients with diabetic nephropathy, retinopathy and neuropathy participated in the study. 24 patients (age 36±9 years (mean±SD), 14 women, diabetes duration 23±9 years, HbA1c 9.1±2.0%) were randomised to receive a structured, problem-oriented, short-term psychotherapeutic intervention (Intervention Group = IG). 22 patients were randomised to the Control Group (CG) (41±10 years, 13 women, diabetes duration 25±10 years, HbA1c 8.7±1.7%), who received medical care in a specialised diabetes university clinic. Each patient could define up to three psychosocial problems (no. 1, no. 2 and no. 3), the severity of the problems was measured on a 1 to 10 graded scale. Severity of disease related symptoms was assessed using the Symptom-Check-List 90-R, quality of life was measured using the IRES questionnaire and depression was evaluated according to the ZERSSSEN questionnaire. Two patients (one in each group) died during the study period. All remaining patients were followed for six months. **Results:** Problem scores were high at baseline in both groups: IG/CG: problem no.1: 7.8±2.0/8.3±1.7; problem no.2: 7.7±2.3/7.6±1.8 and problem no.3: 7.7±2.3/7.4±2.6. At follow-up, all problems were significantly lower in the Intervention Group: IG/CG: problem no.1: 4.3±2.9/6.8±3.0, $p=0.03$; problem no.2: 3.9±2.4/5.8±2.8, $p=0.03$; problem no.3: 4.7±2.4/6.8±2.4, $p=0.02$. HbA1c decreased in the intervention group by 0.6±1.2% and increased in the control group by 0.1±0.7%, $p=0.016$. In patients with HbA1c values above 8%, HbA1c decreased by 1.0±1.2% in the IG and increased by 0.1±0.7% in the CG, $p=0.011$. Severity of disease symptoms, quality of life and depression scores were not significantly different. **Conclusions:** A structured problem-oriented short-term psychotherapeutic intervention decreases the severity of psychosocial problems and improves metabolic control in Type 1 diabetic patients with microvascular complications.

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THE EFFECT OF COPING ON GLYCEMIC CONTROL, COMPLICATIONS, AND SELF-CARE IN TYPE 1 AND TYPE 2 DIABETICS

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Aim: Coping is defined as the behavioral and cognitive efforts used to deal with stressful events. In this study, we aimed to explore how coping with the disease affects the following health outcomes in type 1 and type 2 diabetic adults: treatment adherence, glycemic control, and complications. **Materials and Methods:** Self-report questionnaires were completed by 107 type 2 (age 59.3±11.1) and 89 type 1 patients (age 29.0±11.0) treated with insulin. Coping was measured with the Diabetes Coping Measure, which has four subscales: avoidance, passive resignation, tackling spirit, and lack of integration. Adherence to insulin injections (AII), home blood glucose measurements (AHBM), and diet (AD) was measured with a modified version of the Summary of Diabetes Self-Care Activities Questionnaire. The questionnaire also included questions about acute and chronic complications. Glycemic control was assessed by HbA1c (HPLC-Hitachi). The association between the coping strategies and the outcome variables were tested by partial correlation analyses as well as by obtaining two second-order coping factors and entering those into multiple regression equations predicting the outcome variables. **Results:** Partial correlation analyses (controlling for age, years since diagnosis, marital status, and gender) suggest that coping is an important predictor of outcome, and this relationship is stronger for type 1 patients. With type 1 patients 12 out of 20 correlations (r 's between 0 and .37) were significant, whereas with type 2 only 4 correlations were significant (r 's between 0 and .26). In the regression analyses the factor labeled negative coping was significantly but modestly related to all of the outcome variables in expected directions (β 's between .16 and .33). On the other hand, the positive coping factor was associated with only adherence to diet and adherence to injections, again in the expected directions (β 's between .15 and .17). The interaction terms were not significant for any of the outcome variables except for injection adherence, suggesting that coping is more important in type 1 only for injection adherence. Mediation analyses revealed that adherence only partially mediated the relationship between negative coping and glycemic control. **Conclusions:** Coping with illness is an important factor in diabetes, especially in type 1 diabetes. Therefore, intervention programs may benefit from including a module about coping with diabetes.

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The relationship between insulin-treated diabetes, job characteristics and health outcomes

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Background and Aims: Because employees with diabetes have to cope both with their disease and their work, they are at greater risk of developing health complaints. The aim of our study is to gain more insight into the relationship between job characteristics (workload and decision latitude) and the health status of employees with insulin-treated diabetes.

Materials and Methods: 293 employed insulin-treated persons with type-1 (n=162, response rate 58.9%) and type-2 (n=131, response rate 52.8%) diabetes from three hospitals completed questionnaires which assess job characteristics (Questionnaire on the Experience and Assessment of Work) and health outcomes: fatigue (Checklist Individual Strength), burnout (Maslach Burnout Inventory, 3 subscales) and depressive symptomatology (CES-D). Data were analyzed using t-tests and Pearson correlations.

Results: Compared to Dutch reference values for each questionnaire, employees with diabetes reported a higher workload ($t=4.014$; $p=.000$), more autonomy ($t=-6.754$; $p=.000$), more involvement ($t=-4.139$; $p=.000$), more fatigue ($t=13.462$; $p=.000$), more exhaustion ($t=3.382$; $p=.001$) and a higher level of depression ($t=5.407$; $p=.000$). No differences were found with respect to the burnout components depersonalization ($t=3.03$; $p=.762$) and personal accomplishment ($t=2.016$; $p=.045$). Furthermore, higher decision latitude and lower workload were related to better health outcomes. Comparing the working situation and health status of type-1 and type-2 patients, type-2 patients only report more exhaustion ($t=-2.092$; $p=.037$).

Conclusions: The data indicate high levels of health complaints in diabetic employees, as well as a relationship between job characteristics and health outcomes. This especially emphasizes the need of increasing the decision latitude to promote better functioning at work and to minimize fatigue, burnout and depression.

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A POPULATION BASED STUDY OF FUNCTIONAL HEALTH STATUS AND WELL BEING IN ADOLESCENTS WITH TYPE 1 DIABETES.

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Background and Aims: In adolescents with type 1 diabetes, living with the disease and efforts to obtain and maintain good metabolic control may have negative impact on the patients' emotional and social well being. The objective of the study was to describe functional status and social well being in diabetic adolescents from a well defined geographic area, using both generic and diabetes-specific instruments. **Material and Methods:** All diabetic adolescents 11-18 yrs old attending the pediatric outpatient department at Haukeland University Hospital; Bergen, Norway, were invited to participate. To assess the broad impact of disease and treatment on satisfaction and functioning in daily life, both generic (Child Health Questionnaire, CHQ-CF87) and diabetic (Diabetes Quality of Life Questionnaire, DQOL) measures were used. **Results:** Of a total population of 131 diabetic adolescents, 116 (88.5%) participated. 50% were girls, mean age 14.8 yrs (range 11-18), mean diabetes duration 6.9 yrs (1-16), mean HbA1c 9.3 (6.2-14). Diabetes-related quality of life explained 66% of the variation in general mental health. Neither age, gender, insulin regimen or HbA1c could explain more of the variation. While most of the variance in mental health assessed by CHQ-CF87 is explained by the diabetes-related quality of life, age seems to be an important additional covariate in self-esteem. The effect of age on self-esteem is significantly nonlinear, with lowest self-reported self-esteem being estimated at 15.6 yrs.

Conclusions: Psychosocial support for adolescents with type 1 diabetes seems particular important around the age of 15. Generic instruments like CHQ-CF87 add another dimension to the evaluation of satisfaction and daily functioning in diabetic youths.

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Determinants of Adherence to Preventative Foot Care: a self-regulatory approach.

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Background and Aims: To examine the determinants of reported use of effective, preventative foot care behaviors (PFCB) in patients with diabetic peripheral neuropathy (DN) at high risk of developing foot ulcers. **Materials and Methods:** Use of PFCB was related to demographic factors, disease characteristics, and three types of psychosocial factors: social compliance, beliefs about DN and personality traits. Twelve items assessed use of PFCB. DN was defined by a Vibration Perception Threshold of ≥ 25 volts and a Neuropathy Disability Score of ≥ 3 . Five items assessed social compliance. A theory-based measure assessed 5 areas of common-sense representations of DN (C/DN) and two, emotional responses to DN (E/DN). The 5 cognitive domains were: 1) identity beliefs (correct and incorrect perceptions of DN); 2) time-line (beliefs about DN duration); 3) cause; 4) cure/controllability; 5) consequences and emotions of worry and anger. Cronbach's alphas range: 0.60-0.89. Personality traits were assessed with a 44-item version of the Big Five Inventory (BFI) and negative affect with the Hospital Anxiety and Depression Scale (HADS). **Results:** 188 patients completed psychological assessment (mean age=62 years, diabetes duration =18 years, 71% type 2, 71% male). No BFI or HADS scores were associated with PFCB. Female gender, social compliance and C/DN scores were significantly associated with PFCB before controlling for other factors. Controlling for gender and social compliance, all 5 C/DN beliefs scores and E/DN were significantly associated with PFCB (rp range: 0.17 - 0.29; $p<0.05$). Initial multivariate analysis showed that gender and social compliance accounted for 5.2% of the variance in PFCB, and C/DN and E/DN an additional 12.1%. The two strongest predictors were perceived DN-consequences ($\beta=0.16$; $p=0.042$) and worry about foot problems ($\beta=0.17$; $p=0.043$). Further analyses will examine the possible mediating-moderating effects of C/DN and E/DN on adherence to preventative foot care. **Conclusions:** DN-related cognitions (beliefs) and emotional responses are important determinants of adherence to PFCB and should be addressed when designing behavioural interventions in a group of DN patients at high risk of foot ulceration.

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Islet/Pancreas Transplantation

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INFLUENCE OF LOCAL DELIVERY OF VEGF ON THE VIABILITY OF ENCAPSULATED PANCREATIC RAT ISLETS DURING TRANSPLANTATION

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Background and Aims: Transplantation of encapsulated islets was proposed as a treatment for type 1 diabetes to limit rejection. A significant obstacle to successful transplantation is the low vascularization of the graft during the first few days after implantation leading to the dysfunction and death of islets. The aim of our study was to evaluate the effect of Vascular Endothelial Growth Factor (VEGF) on the angiogenesis of epiploic tissue surrounding the encapsulation device and consequently on the islet survival. **Materials and Methods:** 24 hours after culture, 50 islets immobilized into collagen in the presence or not of VEGF (100 pg/ml) were encapsulated (AN69 membrane, HOSPAL) and implanted in the peritoneal cavity of Wistar rats (n=6). Devices containing collagen supplemented or not with VEGF (100 pg/ml) served as controls. After 7, 14 and 28 days of implantation, encapsulation devices with surrounding epiploic tissue were removed. Histological analysis of this tissue was performed to determine the number and the diameter of vascular buds, the surface of the angiogenic pedicle, and the distance between the device and the buds. Cellular reaction at the membrane surface was analysed by scanning electron microscopy and the cellular type characterized by a phagocytosis test. Morphological aspect of islets was analysed by phase contrast and light microscopy and their functionality evaluated by the measure of insulin secretion in response to glucose stimulation. **Results:** At each step of the study, the number of buds increased by 49.5% in the epiploic tissue surrounding the islet containing device supplemented with VEGF. After 7 days, VEGF increased significantly (i) the bud diameter: 15.4 ± 4.9 vs 5.3 ± 2.4 μm ($p < 0.001$), (ii) the surface of the angiogenic pedicle from 2.82 ± 2.02 to 195.38 ± 45.22 μm^2 ($p < 0.001$). The presence of VEGF decreased significantly the distance between devices and buds: 16.2 ± 5.6 vs 51.6 ± 10.1 μm ($p < 0.001$). Furthermore, the control revealed an increase in buds formation under the influence of VEGF that was emphasized by the presence of islets. The angiogenic effect of VEGF on the epiploic tissue reached a plateau after 14 days after implantation. Membrane surface analysis showed a decrease in macrophage adhesion in presence of VEGF. Islets examination exhibited in presence of VEGF a preservation of their structure without central necrosis. After 7 days, insulin release in response to glucose increased significantly from 33.69 ± 1.52 to 138.84 ± 5.13 $\mu\text{U}/100$ islets when VEGF was added and only from 7.36 ± 0.82 to 26.9 ± 2.5 $\mu\text{U}/100$ islets in its absence ($n=3$, $p < 0.05$). Moreover, 28 days after implantation, islets encapsulated with VEGF exhibited a preservation of their stimulation in response to glucose (121.44 ± 2.01 vs 20.72 ± 1.22 $\mu\text{U}/100$ islets) compared to islets alone (14.2 ± 1.02 vs 5.65 ± 0.99 $\mu\text{U}/100$ islets). **Conclusion:** Stimulation of angiogenesis of epiploic tissue induced by VEGF is associated with a preservation of morphology and functionality of encapsulated islets.

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CHARACTERISTICS AND TRANSPLANTATION OF THE PORCINE NEONATAL PANCREATIC CELL CLUSTERS.

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Background and Aims: Porcine neonatal pancreatic cell clusters (NPCCs) isolated from 1- to 3-day-old pigs (I-A) have cured diabetic nude mice within 8 weeks after transplantation. To shorten the latent period between transplantation of these cells and the reversal of hyperglycemia, we studied NPCCs isolated from 1-month-old pigs (I-B).

Materials and Methods: One- to three-day-old and 1-month-old pig pancreata were cut into fragments, digested by collagenase, cultured for 6 days, and then studied for islet characteristics. Besides, 300 cultured islets were transplanted under kidney capsule of nondiabetic nude mice. At 1 and 3 months after transplantation, the grafts were removed, and insulin content and β -cell mass were measured by radioimmunoassay and point counting morphometry, respectively.

Results: Soon after isolation, I-B was larger than I-A (0.211 ± 0.006 vs. 0.189 ± 0.003 mm^2 , $P = 0.0003$). After 6-day culture, I-B contained more insulin than I-A (6.8 ± 1.4 vs. 2.3 ± 0.2 $\mu\text{g}/150$ NPCCs, $P = 0.02$). However, the stimulation indices of I-A and I-B during static incubation with 500 mg/dl glucose (26.5 ± 3.2 vs. 23.9 ± 1.7) or 500 mg/dl glucose plus 50 mU IBMX (62.2 ± 14.0 vs. 41.9 ± 4.4) were not significantly different. ($P > 0.05$) Furthermore, both I-A and I-B had no first or second phase insulin secretion during sequential perfusion with 100 and 300 mg/dl glucose. The insulin content of the graft at 1 month after transplantation was 0.331 ± 0.031 and 0.333 ± 0.133 μg , and β -cell mass of the graft at 3 months was 0.069 ± 0.022 and 0.067 ± 0.023 mg in recipients with I-A and I-B, respectively. ($P > 0.05$)

Conclusions: NPCCs isolated from 1- to 3-day-old and 1-month-old pigs had different characteristics but similar effects on transplantation.

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SUCCESSFUL INTRAHEPATIC TRANSPLANTATION OF PANCREATIC ISLETS ENCAPSULATED IN ALGINATE MICROCAPSULES IN DIABETIC RATS.

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Background and Aims: The functionality of encapsulated islets transplanted into the peritoneal cavity is limited, based on an insufficient oxygen supply. Therefore, research should focus on finding a transplantation site, which permits closer contact between the encapsulated islets and the bloodstream. In this study, the effect of intraportal injection of encapsulated islets under different experimental settings was evaluated.

Materials and Methods: Therefore 1500 islets each were isolated from Lewis rats, encapsulated in 350 μm alginate capsules of different quality and quantity and transplanted intraportally in streptozotocin-diabetic Sprague Dawley rats.

Results: Concerning the insulin secretion to a glucose challenge no difference could be detected between islets encapsulated in a high purified alginate (A) and those encapsulated in a non-purified alginate (B) (1.57 ± 0.28 vs. 1.6 ± 0.5 ng/islet; n.s.). Islets encapsulated in a high purified alginate (A) show only a slight foreign body reaction after intraportal injection compared to islets encapsulated in a non-purified alginate (B) ($50 \pm 25 \mu\text{m}^2/\text{capsule}$ vs. $345 \pm 59 \mu\text{m}^2/\text{capsule}$; $p < 0.001$). Above a critical capsule mass (> 8000) the intraportal transplantation of encapsulated islets (A) leads to a high intraoperative lethality of the animals (4 from 5) due to occlusion of big portal tracts. In contrast to this, the transplantation of lower yields of capsules leads to no complications (5 from 5 vital). Furthermore, the intraportal injection of encapsulated islets (A) succeed to restore normoglycemia in streptozotocin-diabetic rats ($> 5d$).

Conclusions: For the first time it could be demonstrated, that the intrahepatic transplantation of encapsulated islets without concomitantly immunosuppression succeed to restore normoglycemia in diabetic rats.

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PANCREAS TRANSPLANTATION IN TYPE 2 DIABETES: IS C-PEPTIDE A RECIPIENT SELECTION CRITERION?

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Background and Aims: Incidence and prevalence of secondary complications in patients with type 2 diabetes (DM 2) are increasing steadily. Thirty to 50% of all patients with endstage renal disease entering chronic dialysis are DM 2. Recently manifestation of type 2 diabetes has been observed in young populations and therefore many patients will suffer from diabetic complications much earlier in life. Since the long-term survival of pancreatic grafts in patients with type 1 diabetes are excellent (one-year graft survival 80-90%) Eurotransplant started to consider DM 2 for simultaneous pancreas/kidney transplantation, however excluding those patients with basal C-peptide < 0.8 ng/ml and glucagon-stimulated values three times above baseline. **Methods:** In order to select type 2 diabetic patients for a pancreas/kidney transplantation programme we analyzed C-peptide in 25 non-diabetic patients (age: 61.4 ± 9.8 ; blood glucose: 84 ± 10 mg/dl; BMI 23.3 ± 2.1 kg/m²; HbA1c: $5.0 \pm 0.3\%$) and compared them with 13 insulin-treated type 2 diabetic patients (age: 65.1 ± 10.2 ; blood glucose: 167 ± 43 mg/dl; BMI: 24.3 ± 4.3 kg/m²; HbA1c: $7.4 \pm 1.0\%$). Both groups suffered from end-stage renal disease and were under chronic hemodialysis in average 23 months. **Results:** C-peptide under fasting conditions were very similar in both groups: non-diabetic versus insulin-treated diabetic patients: 9.6 ± 3.6 vs. 9.5 ± 5.5 ng/ml. Postprandial C-peptide levels in non-diabetic dialysis patients were 15.7 ± 6.1 ng/ml indicating good prandial response. In a subgroup of diabetic subjects ($n = 10$) C-peptide was analyzed before 7.1 ± 3.3 ng/ml and after 1 mg glucagon i.v. to 8.8 ± 4.1 ng/ml. Basal blood glucose of 125 ± 23 mg/dl was stimulated by glucagon to 146 ± 18 mg/dl. **Conclusions:** From these data it can be concluded that C-peptide analysis under basal conditions and after glucagon stimulation are not eligible parameters to assess insulin resistance and secretory response in uraemic type 2 diabetes. Consequently C-peptide is not a suitable criterion for pancreas transplantation in insulin-treated DM 2.

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Nephropathy: Genetics and Epidemiology

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CHEMOKINE RECEPTOR CCR5 PROMOTER GENOTYPE ASSOCIATED WITH DIABETIC NEPHROPATHY IN TYPE 2 DIABETIC SUBJECTS

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Background and Aims: Macrophage infiltrations in renal glomerulus of diabetic patients with nephropathy were reported. Recently, chemotactic cytokine receptor 5 (CCR5) promoter 59029G/A polymorphism was identified, and the higher number of the CCR5 on circulating monocyte in the subjects having 59029 A(+) type was observed. Thus, the aim was to evaluate the effect of CCR5 59029 genotype on diabetic nephropathy. **Subjects and Methods:** A total 401 type 2 diabetic subjects showing serum creatinine ≤ 2.0 mg/dl were recruited. CCR5 promoter 59029 G/A genotype was determined by PCR-RFLP. Stage of nephropathy was determined by urinary albumin creatinin ratio (ACR (mg/gCre)) at least two measurements, and classified into normoalbuminuria (ACR < 30, DN0 group), microalbuminuria ($30 \leq \text{ACR} < 300$, DN1 group), and macroalbuminuria (ACR ≥ 300 , DN2 group). **Results:** The allele frequency of 59029A was 54% and the clinical characteristics except ACR were not different between the subjects with or without 59029 A. The frequencies of A(+) type were 75% in the DN0 group, 86% in the DN1 group, and 87% in the DN2 group (DN0 vs. DN1, $P=0.03$, $\chi^2=4.794$; DN0 vs. DN1 and DN2, $P=0.0095$, $\chi^2=6.734$). From logistic regression analysis, 59029A was an independent risk factor for microalbuminuria from normoalbuminuria (O.R.=2.26, $P=0.009$), but not for macroalbuminuria from microalbuminuria. **Conclusions:** CCR5 promoter 59029 A genotype may associate with development of early nephropathy.

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ATRIAL NATRIURETIC PEPTIDE GENE POLYMORPHISMS AND ALBUMINURIA: THE MEXICO CITY STUDY.

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Background and Aims: Atrial natriuretic peptide (ANP) infusion has been found to increase urinary albumin excretion rate (AER) in diabetic patients with microalbuminuria as well as in healthy subjects. Because Scf and BstxI polymorphisms at the ANP gene have been found to be associated with albuminuria in diabetic subjects, we evaluated the relationship between the two polymorphisms and the presence of albuminuria in the Mexico City Study, a population with a very high prevalence of microalbuminuria has been reported. **Materials and Methods:** Allelic and genotype frequencies of these polymorphisms were studied by RFLP analysis with Scf enzyme (wild: A2 vs mutated: A1) and BstxI enzyme (wild: C708 vs mutated: T708) after amplification of two fragments of ANP gene. **Results:** Of 614 subjects (254 men, 360 women), 276 had microalbuminuria (μA) and 60 had established macroalbuminuria (MA); hypertension was present in 71 subjects and type 2 diabetes in 108 subjects. In the whole cohort, the wild/mutated alleles had frequencies of 0.93/0.07 and 0.96/0.04, respectively for Scf and BstxI. Frequency of A2/A1 alleles was 0.93/0.07 in NA subjects; 0.96/0.04 in μA and 0.98/0.02 in MA ($p < 0.05$ $\chi^2=6.9$, for the difference between MA and the other groups). Frequency of C708/T708 alleles was 0.94/0.06 in NA subjects, 0.97/0.03 in μA and 1.0/0.0 in MA ($p=0.03$, $\chi^2=7.5$, for the difference between MA and the other groups). The two polymorphisms were in linkage disequilibrium ($p < 0.0001$). No associations were found between Scf and or BstxI polymorphisms and presence of diabetes, hypertension, stroke and myocardial infarction. In a nominal logistic model adjusting for gender, age, obesity, diabetes, and hypertension, the A1 and T708 allele were independently associated with macroalbuminuria. **Conclusions:** In the Mexico City population, the association between polymorphisms of ANP gene and albuminuria - previously described in Caucasian patients with type 1 or type 2 diabetes - is confirmed despite the very high background prevalence of albuminuria. Thus, these polymorphisms may play a role in protecting subjects against development of albuminuria not only in diabetic subjects but also in the general population.

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POLYMORPHISMS IN THE UCP1-3 GENES ARE NOT ASSOCIATED WITH DIABETIC NEPHROPATHY

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Background and Aims: It was recently shown that normalizing mitochondria superoxide production blocks three pathways of hyperglycemic damage. The human uncoupling proteins, UCP1, UCP2 and UCP3 can uncouple respiration and thereby by reducing the electrochemical potential in the mitochondria and reduce superoxide production. Our aim was to study the association between known UCP1, UCP2 and UCP3 polymorphisms and diabetic nephropathy.

Materials and Methods: 218 diabetic patients with micro- or macroalbuminuria (ALB) and 218 with persistent normoalbuminuria (NORM) with at least 10 years diabetes duration were matched for age, sex, type of diabetes, mean HbA1c levels during the past four years and diabetes duration. 128 of the patients in each group had type 1 diabetes, 88 type 2 diabetes and 2 had unclassified type of diabetes. UCP1 A-G polymorphism at -3826, UCP2 insertion/deletion (I/D) polymorphism and UCP3 C-T polymorphism at -55 were genotyped.

Results: The frequencies of the GG, AG and AA UCP1 genotypes in the NORM vs ALB group were 7.3 % vs. 6.4 %, 37.2% vs. 32.6% and 55.5% vs. 61.0% for AA ($p=NS$). The frequencies of the UCP2 I/D polymorphism were 6.9% vs. 8.3% for I/I, 45.9% vs. 40.3% for I/D and 47.2% vs. 51.4% for D/D genotypes in the NORM vs ALB groups ($p=NS$). Neither did the genotype frequencies for UCP3 C-T polymorphism differ between NORM and ALB patients, i.e. 9.2% vs. 9.7% for TT, 38.5% vs. 41.5% for CT and 52.3% vs. 48.8% for CC.

Conclusions: These data suggest that polymorphisms in the UCP genes are not associated with diabetic nephropathy in this Scandinavian population.

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SA GENE POLYMORPHISM - RELATIONSHIP WITH NEPHROPATHY AND HYPERTENSION IN TYPE 2 DIABETES

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Background and Aims: It has been demonstrated, that high blood pressure remains one of the major factors of progression of various nephropathies. SA gene locus has been identified as being overexpressed in kidneys of spontaneously hypertensive rats (SHR). SA gene PstI polymorphism was found to be associated with the presence of hypertension in non-diabetic subjects. The aim of the study was to test the association between the SA gene PstI polymorphism, the presence of microalbuminuria and/or overt nephropathy, or the presence or absence of high blood pressure in type 2 diabetes.

Materials and Methods: SA gene polymorphism was determined in 693 type 2 diabetic patients. 127 were classified as having overt nephropathy (ON), 323 had microalbuminuria (M), while 243 patients with normoalbuminuria and diabetes duration of at least 10 yrs were considered a control group (N).

Results: SA PstI (A1A1/A1A2/A2A2, %) genotype distributions did not differ between the study groups: N- 83.1/14.4/2.5, M- 79.3/18.9/1.7, ON- 78.7/20.5/0.8. A distortion in genotype distributions was found between hypertensive (HT) and normotensive (NT) subjects (Chi-square test with 2df, $p < 0.05$). Genotype frequencies in the normoalbuminuria group were: (A1A1/A1A2/A2A2, %): HT- 86.8/12.5/0.7, NT- 77.8/17.2/5.0, while in the microalbuminuria+overt nephropathy group: HT- 81.0/16.7/2.3, NT- 75.0/25.0/0.0. Moreover, subjects homozygous for the A2 allele had lowest blood pressure values (systolic and diastolic), homozygous for the A1 allele - highest blood pressure, with heterozygotes presenting intermediate values.

Conclusions: The SA gene PstI polymorphism was found to be associated with high blood pressure in type 2 diabetic subjects, but with no impact on the development of incipient or overt nephropathy in these subjects.

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GNB3 POLYMORPHISM ALLELE TRANSMISSION TO OFFSPRING AFFECTED WITH END-STAGE RENAL DISEASE OR DIABETIC NEPHROPATHY

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Background and Aims: Arterial hypertension is an independent predictor of progression of nephropathies of various etiologies. It has been pointed, that parents of type 1 diabetic patients with nephropathy have higher blood pressure values, and more frequently have hypertension, than parents of patients without nephropathy. It is thus postulated, that genetics of hypertension, diabetic nephropathy and progression of chronic renal failure may overlap. Recent reports have suggested, that a molecular variant of the guanidine nucleotide binding protein β_3 subunit (GNB3) - C825T is associated with the increased risk of the development of hypertension. The aim of this study was to assess whether GNB3 C825T polymorphism is associated with the increased risk of the development of end-stage renal disease in non-diabetic patients, or diabetic nephropathy in type 1 diabetic subjects. The hypothesis was tested using a family-based study design - transmission disequilibrium test.

Materials and Methods: GNB3 polymorphism was determined in: (1) 200 non-diabetic patients with end-stage renal disease, either conservatively treated (with creatinine clearance <30 ml/min), or submitted to chronic renal replacement therapy (hemo- or peritoneal dialysis); (2) 47 type 1 diabetic patients with end-stage renal disease, as well as in 28 microalbuminuric or overt proteinuric type 1 diabetic patients with normal renal function. GNB3 polymorphism was also determined in both parents of the examined patients, and observed and expected (random) transmissions were compared using a Mac Nemar's statistics.

Results: C/T allele transmission (%) in non-diabetic, end-stage renal disease patients was 48/52, in type 1 diabetic patients with end-stage renal disease - 50/50, while in type 1 diabetic patients and microalbuminuria or overt proteinuria - 44/56, and these differences were not significantly different in McNemar's test.

Conclusions: Results of the present study provide evidence against major impact of the GNB3 polymorphism on the development of nephropathy in type 1 diabetic patients, as well as end-stage renal disease in non-diabetic subjects.

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ALLELE 5 OF THE ANGIOTENSINOGEN MICROSATELLITE MARKER IS ASSOCIATED WITH DIABETIC NEPHROPATHY.

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Background and aims: Both lifestyle related factors such as smoking, hypertension and elevated HbA1c and genetic factors are important for the development of diabetic nephropathy (DN). Several genetic polymorphisms in the renin-angiotensin system have been identified and suggested to be associated with DN. The aim of this study was to investigate if genetic markers located near the angiotensinogen (Ang) gene, the angiotensin II type-1 receptor (AGTR1) gene or the ACE-gene give an increased risk for developing DN, and if any of these polymorphisms show interaction with smoking and poor metabolic control.

Materials and methods: The genetic markers we focused on were the insertion/deletion (I/D) polymorphism in the ACE-gene, the M235T polymorphism in the Ang-gene and two microsatellite markers, one located near the Ang-gene and the other located near the AGTR1-gene. DNA samples were collected from 190 type-1 diabetic patients. Patients with diabetes duration of 15 to 20 years or more, without albuminuria, were considered as controls (n=90). Albumin excretion rate 20-200 μ g/min was considered as incipient nephropathy (n=63) and albumin excretion rate over 200 μ g/min was considered as overt nephropathy (n=37). Smoking habits and HbA1c values were obtained from questionnaires and hospital records.

Results: Allele 5 of the Ang-gene microsatellite was significantly associated with an increased risk for DN, crude OR=2.27 (95% CI 1.04-4.95). Smoking was associated with an increased risk for DN, OR=2.46 (95% CI 1.23-4.93). Among smokers allele 5 was still significant but did not increase the OR, OR=1.32 (95% CI 1.09-1.60), this was also the case among patients >75th percentile in HbA1c, OR=1.54 (95% CI 1.12-2.12). In this material, no association between DN and genotype was found with the I/D or M235T polymorphisms or with the AGTR1-gene microsatellite.

Conclusions: Allele 5 of the Ang-gene microsatellite marker is associated with increased risk for DN. No interactions between this allele and smoking or poor metabolic control were found.

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Impact of renal impairment on the death rates of people with diabetes

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Background and Aims: To investigate, in people with diabetes, differences in all cause mortality between those with and without renal impairment. **Materials and Methods:** All people with known diabetes, in South Tees, UK, alive on the 1st January 1994 were identified, tagged with the UK Office for National Statistics and followed up until 31st December 1999. Data on mortality was collated from death certificates. A threshold for creatinine of 150 μ mol/l was used to divide the cohort into those with normal renal function and those with renal impairment, at the start of the study. Age specific death rates were calculated for each group, with 95% confidence intervals, using a person years method. **Results:** Overall 104 of 153 (68.0%) of people with renal impairment died compared to 1101 of 4688 (23.5%) people with normal renal function. People with renal impairment made up 3.2% of the diabetic population but accounted for 8.6% of the deaths. Of 104 deaths in people with renal impairment only 5 had renal disease certified as the underlying cause of death, cardiovascular disease accounted for 75% of deaths, compared to 58% in those people with normal renal function, $p=0.001$. Death rates per 1000 person years (95% CIs) are shown below:

Diabetes Type	Age Band	Male		Female	
		Creatinine $<150 \mu$ mol/l	Creatinine $>150 \mu$ mol/l	Creatinine $<150 \mu$ mol/l	Creatinine $>150 \mu$ mol/l
1	20-39	3 (0 to 6)	0	2 (1 to 5)	0
	40-59	19 (11 to 27)	65 (24 to 107)	15 (7 to 24)	128 (67 to 186)
	60-79	54 (34 to 74)	131 (37 to 224)	82 (61 to 102)	343 (55 to 632)
2	40-59	21 (17 to 25)	95 (61 to 108)	10 (8 to 14)	403 (0 to 836)
	60-79	54 (51 to 57)	174 (145 to 203)	49 (46 to 53)	232 (164 to 299)
	80+	143 (132 to 153)	373 (246 to 501)	132 (125 to 138)	189 (136 to 242)

Conclusions: People with and diabetes and renal impairment account for a disproportionate number of deaths from the total diabetes population. Reliance on death certification data to estimate the contribution of renal failure to mortality will lead to significant underestimation of the impact of renal failure. Creatinine level is a simple, cheap, and easily available, marker that identifies a subgroup of people with diabetes who have increased mortality rates, compared to the high death rates of people with diabetes.

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THE RESULTS OF LONG-TERM MICROALBUMINURIA SCREENING IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES.

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The aim: to analyse the results of prospective study of urinary albumin excretion rate (UAER) in diabetic children and adolescents. **Patients:** 528 children (261 boys, 267 girls), aged 3 - 25 years (522 i.e. 98.8% under the age 19 years). Diabetes duration at the beginning of the study 1 month to 20 years (317 children i.e. 60% were observed from the onset of diabetes). The period of follow-up was 1 - 13 years (median 5.4 years) **Methods:** UAER was determined by radioimmunoassay in overnight urine collections. Microalbuminuria (MA) was defined as UAER $>15 \mu$ g/min in at least two of three urine samples. **Results:** I. At the onset of the study MA was found in 48/528 (9%) children (21 boys and 27 girls). The frequency of MA increased significantly with age (we didn't observed MA in youngest group - 0-4 years) and with diabetes duration and was as follows: 0-4 years - 6.6%, 5-11 years - 9.5%, 12-17 years - 11.8% and >17 years of diabetes - 30.8%. In children who developed diabetes under the age 5 years MA was found only in the pubertal age (>11 years). Patients with MA had significantly higher mean HbA1c (9.1 vs 9.8%, $p<0.01$) and HbA1c at the beginning of the study (11.12% vs 12.17%). The risk factors for MA development were: age >11 years (OR 13.3, 95%CI 2.8-64), diabetes duration (OR 1.1, 95%CI 1.1-1.3) and mean HbA1c (OR 1.3, 95%CI 1.1-1.7) At follow-up MA persisted >3 years in 12 of 48 patients with MA at the onset of the study II. During the follow-up of 443 patients with normal UAER at the onset, normoalbuminuria was observed in 318 (71.8%) of diabetic patients, MA was detected only once (in one urine sample) in 95 (21.5%) of children. In 20 (4.5%) of patients MA was observed for 1-2 years and in 10 patients (2.2%) persisted for at least 3 years. In comparison to normoalbuminuric children, patients with persistent microalbuminuria had significantly worse long term glycemic control (higher mean HbA1c from the onset of diabetes 9.8% vs 9.0%). Additionally, they had significantly higher UAER at the beginning of the study. The highest incidence of persistent MA was found in patients with diabetes onset at the age 5 - 11 years.

Conclusions: children and adolescents with type 1 diabetes should be screened for microalbuminuria early in the course of the disease and they required good glycemic control to minimise the risk of diabetic nephropathy development.

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Albuminuria in a population based study of Danish patients with type 1 diabetes mellitus: Prevalence, clinical characteristics and risk factors

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Background and aims: The urinary albumin excretion rate (UAER) has proved to be one of the most important prognostic factors in the secondary prevention of several chronic complications of type 1 diabetes mellitus. Thus, it is our aim to present data regarding prevalence, clinical features and possible predictors of albuminuria in a cross sectional study of a well defined cohort of patients with type 1 diabetes mellitus.

Materials and methods: The study is population based and the patients identified by means of insulin prescriptions with a degree of ascertainment of 97%. The population consisted of 681 patients with type 1 diabetes mellitus, 281 females and 357 males. Median age was 34 years (range 7-79), median duration of diabetes 17 years (range 0-63). The method for determination of UAER was based upon three overnight urine collections.

Results and conclusion: We found a prevalence of microalbuminuria (95% CI) of 12% (9.6, 14.6), and for macroalbuminuria of 8.6% (6.4, 10.8). The prevalence of microalbuminuria increased by duration of diabetes until 20 years of duration, where after it remained constant. A steady increase in the prevalence of microalbuminuria by increasing HbA_{1c} levels as well as by increasing diastolic and systolic blood pressure was observed. The blood pressure was within the normal range in patients with microalbuminuria. Glomerular filtration rate as estimated from measurement of creatinin clearance was stable in patients with microalbuminuria but decreased in patients with overt nephropathy. An analysis of possible risk factors in a multivariate model revealed that age, duration of diabetes, blood pressure, HbA_{1c}, serum-cholesterol and current smoking were all significantly associated to increased levels of albuminuria.

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POPULATION SCREENING TO DETECT NEPHROPATHY IN SINGAPOREANS WITH DIABETES OR HYPERGLYCEMIA

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Background and Aims: A population-based program for the early detection and intervention of type 2 diabetes (DM), hypertension (HTN) and renal disease was initiated in Singapore. One element of this program was to determine the level of glycemic control and blood pressure control in individuals with known DM and in individuals at risk for DM. **Materials and Methods:** Singaporeans ≥ 18 years old were screened by questionnaire (demographics, prior medical history including type 2 diabetes (DM) and hypertension (HTN), family history and lifestyle habits), BP according to established national guidelines, random capillary blood glucose (CBG) and urinalysis from January 2000 to September 2000. Nephropathy was defined as proteinuria (PR) of ≥ 30 mg/dL or $\geq 1+$ on dipstick analysis. Hyperglycemia (HPG) and systolic hypertension (SHTN) were defined as a CBG ≥ 8.9 mmol/L and systolic BP ≥ 130 mmHg, respectively. **Results:** Two thousand subjects completed the screening (53% M/47% F). Seventy-six percent were Chinese, with the remainder Malay, Indian or other. The mean age was 40.6 years. Known disease was found in 64 cases (3.2%) of whom 46 had DM and 18 had DM and HTN. In those with both HTN and DM, 67% had HPG and 72% had SHTN. Among those with known DM alone, 50% had HPG and 50% had SHTN. Eleven percent of those with both HTN and DM had significant PR, reflecting previously undetected nephropathy. Undetected HPG was present in 38 subjects (1.9%); an additional 38 subjects (1.9%) were found to have undetected HPG with SHTN. Among those with HPG alone, 2.8% had significant PR. Among those with both HPG and SHTN, 12.1% had PR. This was significantly higher than the prevalence of PR among those with neither HPG nor SHTN (1.8%, $p < 0.0001$). **Conclusion:** Individuals known to have DM with or without HTN were found to have elevated blood glucose and systolic blood pressure, associated with a high prevalence of previously undetected nephropathy. Furthermore, a high percentage of individuals with previously undetected hyperglycemia, with or without SHTN were found to have established renal disease.

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Nephropathy: Pathology

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RENAL STRUCTURE IN TYPE 2 DIABETIC PATIENTS WITH ALBUMINURIA

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Background and Aims: Renal biopsies were obtained from Type 2 diabetic patients with elevated albumin excretion. The aim was to obtain quantitative structural data for the study of correlation with clinical findings.

Materials and Methods: Biopsies from 27 patients were analysed and data compared with those obtained in 13 non-diabetic cases. Stereological methods were applied by light- and electron microscopy.

Results: Diabetic patients showed quantitatively markedly expressed diabetic glomerulopathy but also an increase in glomerular volume, prevalence of glomerular occlusion and in interstitial volume fraction. A significant correlation was not observed between the degree of interstitial and glomerular involvement. The glomerular hypertrophy is interpreted as a compensatory phenomenon, leading to preservation of filtration surface in the open glomeruli. Close correlation was seen between glomerulopathy and glomerular function, and also with the stage of retinopathy. Signs of non-diabetic glomerulopathy were not observed, but various atypical ultra-structural changes accompanying the advanced stages are illustrated.

Conclusions: Our present findings correspond to data from Type 1 diabetic patients. It is emphasised that all compartments of the kidney are affected by the diabetic state. It is suggested that the interstitial and glomerular lesions are influenced by mutually different factors.

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SIGNIFICANT CORRELATION OF TRANSFORMING GROWTH FACTOR β WITH METABOLIC CONTROL AND SYSTOLIC BLOOD PRESSURE IN TYPE 2 DIABETIC PATIENTS

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Background and Aims: Transforming growth factor beta (TGF- β) is a cytokine, which plays a major role in the regulation of cell proliferation and seems to be an important determinant of diabetic glomerular injury. This hypothesis has been supported by the detection of increased concentrations and enhanced expression of TGF- β in and from the target tissues of diabetic long-term complications.

Materials and Methods: To analyse the association of urinary TGF- β excretion with degree of hypertension and quality of metabolic control we studied this correlation in a type 2 diabetic study population with normo-, micro- and macroalbuminuria. In this cross sectional analysis urinary levels of TGF- β were measured in 39 type 2 diabetic patients, 27 male and 12 female, age: 63 ± 9 years, duration of diabetes: 12 ± 7 years.

Results: We found a significant correlation of urinary TGF- β excretion rate with systolic blood pressure ($r = 0.54$, $p < 0.0001$), with metabolic control ($r = 0.33$, $p < 0.03$) and with urinary albumin excretion rate ($r = 0.43$, $p < 0.007$). The correlation with glomerular filtration rate was also significant ($r = 0.42$, $p < 0.01$). We could not demonstrate any significant correlation with age, diabetes duration and sex. In contrast to published study results from type 1 diabetic patients we could not detect an association between ACE-inhibitor medication and TGF- β excretion rates. This implicates that rather the level of blood pressure and not the kind of medication seems to be essential in treating hypertension in diabetic patients.

Conclusions: TGF- β might be an useful indicator of glomerular injury in longterm follow up in type 2 diabetic patients.

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INTIMAL MEDIA THICKNESS DOES NOT DIFFER IN TYPE 2 DIABETIC PATIENTS WITH DIFFERENT DEGREES OF ALBUMINURIA.

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Background: Intimal media thickness (IMT) of common carotid artery (CCA) has been widely used as an index of atherosclerosis. Diabetes and diabetic nephropathy are conditions associated with accelerated atherosclerosis. **Aim:** The study was done to assess the IMT in type 2 diabetic patients with different degrees of albuminuria to see whether albuminuria influenced IMT. **Material and methods:** IMT of CCA was measured in 264 type 2 diabetic subjects with ≥ 5 years diabetes duration, using high resolution B mode ultrasonography. Subjects with normoalbuminuria ($<30 \mu\text{g}$ albumin /mg creatinine) (NAU) (n=91), microalbuminuria (30-300 μg albumin /mg creatinine) (MAU) (n=92) and clinical proteinuria ($>500 \text{ mg/day}$) (Prot) (n=90) were studied. Results were compared with 99 age matched non-diabetic subjects. All subjects had measurements of anthropometry, biochemical parameters including lipids and HbA1c and blood pressure. Statistical comparisons between groups were done using students t' test and multiple linear regression analysis. **Results:** The mean age-adjusted IMT value in diabetic subjects was significantly higher ($0.88 \pm 0.3 \text{ mm}$) than in non-diabetic subjects ($0.57 \pm 0.34 \text{ mm}$) ($P < 0.001$). Mean IMT in NAU ($0.87 \pm 0.26 \text{ mm}$), MAU ($0.90 \pm 0.33 \text{ mm}$) and Prot ($0.86 \pm 0.39 \text{ mm}$) were not significantly different from each other. Multiple regression analysis showed that male gender, age, diabetes and total cholesterol were independently associated with IMT. Duration of diabetes, hypertension and HbA1c did not show independent association with IMT although they were significantly higher in diabetic subjects. **Conclusion:** Diabetes was a major contributing factor for higher IMT. However, within the diabetic subjects, presence of albuminuria did not have additional influence on the IMT.

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Different effect of melatonin and glutathione on adenosine metabolism in rat glomerular mesangial cells cultured under high glucose conditions
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Glomerular mesangial cells play a major role in glomerular haemodynamics, but they are also considered as antigen-presenting cells participating in immune response. It is the site of typical proliferative lesions in diabetic glomerulopathy. Adenosine, a local hormone, produced by mesangial cells is a metabolic regulator of renal blood flow, capable of decreasing glomerular filtration rate (GFR), but exerts also immunosuppressive, antiproliferative and anti-inflammatory properties. **Aim:** Since it was well established that antioxidants confer protection against increased oxidative stress that occurs in diabetes, in this work we have looked for the potential effectivity of melatonin and glutathione to ameliorate adenosine metabolism. Melatonin exerts immunoenhancing effect, mediated through specific receptors and cellular signals, but it is also an efficient antioxidant, acting by itself, rather than through specific binding sites. **Methods:** Glomerular mesangial cells obtained from collagenase treated glomeruli, isolated from renal cortex of Sprague-Dowley rats were cultured in RPMI-1640 medium at 37°C , supplemented with 10% FCS and 20mmol HEPES pH=7.2. After two passages confluent cells were grown under high glucose conditions (30mmol/l) as a model of diabetic microenvironment. The activity of adenosine metabolizing enzymes: 5'-nucleotidase (5'-NU) responsible for its production and adenosine deaminase (ADA) - responsible for its degradation were investigated during exposure to melatonin (10^{-5} mol) and reduced glutathione (GSH 10^{-2} mol). **Results:** Hyperglycaemic conditions led to decreased adenosine production via 5'-NU (4.40 ± 1.27 vs $11.29 \pm 1.22 \text{ U/g prot.}$ $p < 0.001$) of control cells cultured under normoglycaemic (5mmol/l) conditions, that resulted in its decreased removal via ADA (13.20 ± 1.01 vs control $23.75 \pm 2.01 \text{ U/g prot.}$ $p < 0.01$). This event may contribute to the loss of afferent arteriolar contractility, mesangioproliferative activity and cytoskeletal dysfunction. When control cells were exposed to melatonin (from 10^{-6} to 10^{-4} mol/l) 5'-NU activity increased (from 13.90 ± 1.22 to 24.93 ± 3.30 $p < 0.05$), while ADA activity was not changed significantly, implicating on increased adenosine production. Similar results were obtained after exposure to GSH (from $0.5 \cdot 2 \times 10^{-2} \text{ mol}$) for 5'-NU (from 13.95 ± 1.34 to 38.23 ± 3.5 $p < 0.001$) and for ADA (25.2 ± 3.15 to 25.11 ± 2.40). But when hyperglycaemic cells were exposed to melatonin 5'-NU decreased significantly (3.25 ± 1.01), but ADA did not change significantly implicating on decreased adenosine metabolism, and observed effect may be explained by increased protein kinase C signaling pathway by hyperglycaemia, which can decrease melatonin action. GSH ameliorated adenosine metabolism (5'-NU was 9.32 ± 1.22 and ADA 15.9 ± 2.26). **Conclusion:** Presented results confirm recent findings that the potential value of melatonin supplementation during diabetes is still uncertain and awaits future investigation.

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EFFECT OF APO E2-REMNANT LIPOPROTEINS ON PKC ACTIVITY AND TGF- β AND PROTEIN MATRIX SYNTHESIS IN MESANGIAL CELLS

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Background and Aim: Lipid abnormalities are associated with macroangiopathy and microangiopathy including diabetic nephropathy (DN). Apolipoprotein (apo) E2 causes an increase of remnant lipoproteins in plasma. We previously reported that apo E2 allele contributes to the progression of DN (Atherosclerosis 107:203-211,1994, Clin Genet 48:288-292,1995). Recently this finding has been supported in Caucasian diabetic patients (Diabetes, Diabetes Care 1998). However, the mechanism by which apo E2 promotes DN is not known. In this study we examined the effect of apo E2-remnant lipoproteins on PKC activity and TGF- β and protein matrix synthesis in human mesangial cells (HMCs).

Methods: Plasma samples were collected from Type 2 diabetic patients with apo E2/2, E3/2 or E3/3. IDL (density 1.006-1.019) was isolated from the plasma by ultracentrifugation. Normal HMCs were incubated for 24 hrs with IDL (50 mg/ml protein) in the medium. After the incubation, PKC activity was assayed using BIOTRAK, and the concentration of TGF- β and protein matrix in the medium was measured by ELISA method.

Results: IDL from diabetic patients with apo E2/2 or E3/2 stimulated PKC activity more significantly than that with apo E3/3 (343% or 174% vs 100%) after incubation with HMCs. Furthermore, IDL from diabetic patients with apo E2/2 or E3/2 caused a significant increase in TGF- β , type IV collagen and fibronectin synthesis by HMCs.

Conclusions: Apo E2-remnant lipoproteins from diabetics stimulate PKC activity and TGF- β and protein matrix synthesis in HMCs, and may cause mesangial stretching, glomerulosclerosis and DN. Apo E2 contributes to the progression of DN at least partly through remnant lipoproteins.

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Lovastatin inhibit Insulin-like growth factor-1 expression on cultured mesangial cells

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Background and Aims: Insulin-like growth factor-1 (IGF-1) is a marker of glomerular hypertrophy in the early stage of diabetic rats. HMG-CoA reductase inhibitor have suppressive effect on transforming growth factor- β 1 (TGF- β 1) and cultured mesangial cells. This study examined the effect of lovastatin on IGF-1 expression of mesangial cells.

Materials and Methods: Human mesangial cells were cultured in DMEM containing low glucose (5.6mM), high glucose (30mM) with lovastatin (10 μM) for 48 hours, respectively. IGF-1, TGF- β 1 mRNA expression of cultured mesangial cells were tested by RT-PCR and TGF- β 1, fibronectin, laminin, type IV collagen in supernatant were determined using RIA and ELISA techniques.

Results: TGF- β 1, fibronectin, laminin, type IV collagen and IGF-1, TGF- β 1 mRNA expression were increased in the mesangial cells cultured in high glucose as compared with low glucose. Both TGF- β 1 and IGF-1 mRNA expression in cultured mesangial cells in high glucose condition were suppressed by lovastatin. In the cultured mesangial cells with high glucose and lovastatin, the decrease of TGF- β 1, fibronectin, laminin and type IV collagen proteins were also observed as compared with cells in high level of glucose without lovastatin.

Conclusions: Lovastatin suppresses fibronectin, laminin, type IV collagen products and expression of IGF-1, TGF- β 1 mRNA in cultured mesangial cells. This study suggests that the preventive effect of lovastatin on diabetic nephropathy result not only from the suppression of TGF- β 1, but also from its inhibitory effect on IGF-1 expression.

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TRANSFORMING GROWTH FACTOR- β 1 (TGF- β 1) COUNTERACTS THE PROTECTIVE EFFECT OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 2 (VEGFR-2) IN HUMAN GLOMERULAR ENDOTHELIAL CELLS (GENC).

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Background and aim: two major VEGF receptors have been identified and cloned: VEGFR-1 (Flt-1) and VEGFR-2 (KDR/flk-1). They are mostly expressed in endothelial cells. In the rat VEGFR-2 has been identified in glomerular endothelial cells and a role in diabetic nephropathy has been proposed. In human diabetic nephropathy the role of VEGF and its receptors have not been clearly demonstrated. We investigated the presence of VEGF receptors and their modulation in human glomerular endothelial cells with particular regard to the linkage with TGF- β 1. **Materials and Methods:** for binding experiments GENC have been grown at confluence and serum free in 24-well plates for 24h then for 24h with serum free and stimuli (TGF- β 1 100nM) performing families of displacement curves between [125 I]VEGF and increasing concentrations of cold VEGF. RT-PCR was performed by using primers previously designed for VEGF receptor subtypes. For the proliferation assay GENC were seeded in 24-well plates for 24 h in serum free and incubated with appropriated stimuli and 3 H-Thymidine. Nitric oxide (NO) synthase has been quantified with a commercial kit evaluating the conversion of [3 H]-arginine to [3 H]-citrulline. **Results:** using scatchard analysis we have shown the presence of VEGF receptors in GENC. The mathematical model suggested the presence of one class of binding sites with a binding capacity of 29.27 ± 12 fmoles/1E6 cells and a Kd of 0.35 ± 0.19 nM. The receptor number was not significantly affected by the presence of heparin (0.1ng/ml); however, a slight decrease of about 14% of binding sites was observed. RT-PCR has shown the predominant presence of VEGFR-2 with no apparent signal for VEGFR-1. VEGF was able to stimulate (38% vs control) thymidine incorporation and, in preliminary results, the production of NO (55% vs control). The mitogenic action of VEGF was counteracted by the addition of TGF- β 1. Furthermore, mathematical analysis of multiple homologous curves for [125 I]VEGF indicated that the TGF- β 1 treatment induces a decrease (80%) in VEGFR-2 specific binding capacity without changing the binding affinity. **Conclusions:** we demonstrated for the first time, the presence of VEGFR-2 in GENC. Since VEGF induced a proliferative effect and a direct control of NO production, these data suggest that VEGF could act as survival factor for GENC and this effect is inhibited by TGF- β 1.

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Protective effects of Lithospermate B on diabetic nephropathy

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Background and Aims: ROS generation and PKC activation have been observed in mesangial cell cultured under high glucose and in the kidneys of experimental diabetic animals. Lithospermate B (LAB), a recently isolated component of *S. miltiorrhiza*, has beneficial effect on renal function of nephrectomized rats possibly through the reduction of ROS generation. In the present study, we have investigated whether LAB can ameliorate diabetic nephropathy.

Materials and Methods: To in vitro system, murine mesangial cells were grown in DMEM media. Near confluent mesangial cells were incubated with serum-free media for 24 hours to arrest and synchronize the cell growth. Then, the media was changed to serum-free DMEM containing different concentrations of glucose in the presence or absence of LAB. After LAB treatment, ROS generation, PKC activity, and TGF- β 1 and fibronectin protein synthesis were detected.

To in vivo system, four experimental groups were formed: STZ-induced diabetic rats (STZR; n = 6), age-matched control rats (CR; n = 6), diabetic rats treated with LAB (LAB + STZR; n = 6), and control rats treated with LAB (LAB + CR; n = 6). After treatment of LAB for 16 weeks, 24 hours urinary albumin excretion was measured.

Results: LAB inhibited ROS generation (normal control mesangial cell: 100%, 100uM H₂O₂ treated mesangial cell: $148.64 \pm 9.25\%$, 100uM H₂O₂ + 20ug/ml LAB treated mesangial cell: $53.72 \pm 8.49\%$, all p < 0.05) and PKC activation (normal control mesangial cell: 100%, high glucose treated mesangial cell: $148.35 \pm 19.85\%$, 10ug/ml LAB + high glucose treated mesangial cell: $35.46 \pm 3.98\%$, all p < 0.05) in mesangial cells cultured under high glucose. And effectively protected the progression of albuminuria in diabetic rats (control diabetic rat: 221 ± 34 mg/day, LAB treated diabetic rat: 43 ± 33 mg/day). At the general characterization of experimental rat, plasma glucose level was not effected by LAB between STZR and LAB + STZR. Kidney weight / 100g body weight was increased at STZR compared with normal CR, and decreased at LAB + STZR compared STZR.

Conclusions: This study provides an evidence that LAB, an active components of *S. miltiorrhiza* radix, could be a new therapeutic agents for the treatment of diabetic nephropathy.

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Metabolic vs. hemodynamic factors in the pathogenesis of diabetic glomerulopathy: studies in the Milan rat model

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Background and Aims: Rats of the Milan normotensive strain (MNS) develop an age-dependent glomerulosclerosis (GS), although they maintain normal blood pressure (BP) levels, whereas rats of the Milan hypertensive strain (MHS), developing moderate form of arterial hypertension (AH), are resistant to GS, possibly due to hypertrophy of intrarenal arteries protecting glomerular capillaries towards increased BP. This study was aimed at testing the hypothesis that diabetes accelerates the development of GS in the MNS and/or remove protection from renal disease in the MHS. **Materials and Methods:** MNS and MHS rats (aged 3 months) were rendered diabetic (D) by streptozotocin (55 mg/kg i.v.) and killed 6 months later, together with age-matched nondiabetic controls (ND) for the assessment of metabolic control, BP and renal function and structure. Three-month-old rats were also sacrificed for time 0 evaluation. **Results:** BP was normal in MNS and elevated in MHS rats and was not influenced by diabetes. Serum creatinine and proteinuria increased in MNS, with no difference between D and ND (49.3 ± 7.2 vs. 44.5 ± 3.7 μ mol/l and 802 ± 125 vs. 762 ± 120 mg/die), whereas they did not change in both MHS-D and ND (34.0 ± 5.7 vs. 33.1 ± 6.8 and 13.4 ± 4.3 vs. 16.7 ± 2.1). Glomerular disease was detected in the MNS, with diabetes accelerating lesions typical of diabetes, i.e. increased glomerular area (11252 ± 886 μ 2 in D vs. 9875 ± 691 in ND, p < 0.01), fractional mesangial area ($9.4 \pm 1.8\%$ in D vs. $4.9 \pm 0.9\%$ in ND, p < 0.001) and GBM thickness (275 ± 15 nm in D vs. 223 ± 13 nm in ND, p < 0.05), but not of those characteristic of the age-dependent GS of this rat strain, i.e. glomerular sclerosis ($18.0 \pm 12.3\%$ in D vs. $20.6 \pm 10.3\%$ in ND) and scoring of tubulointerstitial damage (2.29 ± 1.10 in D vs. 2.14 ± 0.71 in ND). In contrast, the MHS rats showed no renal disease, as assessed by the above morphometric parameters, even after 6 months of uncontrolled diabetes; this was associated with renal arteriole hypertrophy in both D and ND groups, as shown by the increased thickness (7.7 ± 0.8 and 7.4 ± 0.6 μ vs. 6.2 ± 0.5 and 6.4 ± 0.4 in the MNS), ratio to lumen and cross area of the media. **Conclusions:** The fact that metabolic injury accelerates kidney disease in the GS-prone strain by superimposing diabetes-specific lesions, but is not able to produce renal lesions in the GS-resistant strain, due to the hypertrophy of intrarenal arteries, points to the importance of hemodynamic vs. metabolic factors in the pathogenesis of diabetic glomerular disease. In addition, it further supports a role for genetic predisposition.

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Combination of Simvastatin and Cilazapril have preventive effect on diabetic nephropathy in Diabetic Rat and Cultured Mesangial Cells

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Background and Aims: Diabetic nephropathy is a leading cause of end-stage renal disease and is characterized by excessive deposition of extracellular matrix (ECM) proteins in the glomeruli. Transforming growth factor- β 1 (TGF- β 1) is the major mediator of excessive accumulation of ECM proteins in diabetic nephropathy through upregulation of genes encoding ECM proteins as well as downregulation of genes for ECM-degrading enzymes. It has been shown that lovastatin, an inhibitor of 3-hydroxy-methylglutaryl CoA reductase, delays the onset and progression of different models of experimental nephropathy. On the other hand, cilazapril, an inhibitor of angiotensin converting enzyme (ACE) can decrease albuminuria and delay progression of diabetic nephropathy as well.

Materials and Methods: To evaluate the effect of simvastatin (3.6mg kg⁻¹.d⁻¹ by gavage) and combination of simvastatin and cilazapril (1.0mg kg⁻¹.d⁻¹ by gavage) on the development and progression of diabetic nephropathy, streptozotocin-induced diabetic rats were studied for 8 weeks. To elucidate the mechanisms of the renal effects of simvastatin and cilazapril, human mesangial cells were cultured in control (5.6 mM) or high (30mM) glucose with simvastatin (10 μ M), cilazapril (10 μ M) or both.

Results: Compared with untreated diabetic rats, both simvastatin and cilazapril significantly decreased urine albumin/creatinine excretion rate (p < 0.01), suppressed TGF- β 1 mRNA expression (p < 0.01), TGF- β 1, fibronectin, laminin and type IV collagen levels (immunohistochemistry) in renal cortex, especially in combination group. Under high glucose, TGF- β 1 mRNA and TGF- β 1, fibronectin, laminin, type IV collagen proteins were upregulated. These high glucose-induced changes were suppressed by either simvastatin alone or combination of simvastatin and cilazapril.

Conclusions: These results suggest that simvastatin has a direct cellular impact independent of a cholesterol-lowering effect and delays the onset and progression of diabetic nephropathy through suppression of glomerular expression of TGF- β 1 and reduction of fibronectin, laminin and type IV collagen proteins in renal cortex. Combination of simvastatin and cilazapril can increase the effect by different mechanisms.

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ATRIAL NATRIURETIC PEPTIDE INCREASES URINE ALBUMIN EXCRETION IN MICROALBUMINURIC SUBJECTS WITH TYPE 2 DIABETES MELLITUS.

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Background: Atrial natriuretic peptide (ANP) has been implicated in the pathogenesis of microalbuminuria in type 1 diabetes mellitus. This study aimed to demonstrate the albuminuric effect of atrial natriuretic peptide in patients with type 2 diabetes. **Methods:** 7 male patients with microalbuminuric type 2 diabetes (age 50 ± 8.8 , duration of diabetes 6.86 ± 2.67 yrs) underwent a two limb, single blind, placebo controlled study. After overnight fast, patients were fluid loaded orally (20ml/kg tap water and then replacing urinary losses) to achieve steady state diuresis. Plasma glucose was maintained in the euglycaemic range using intravenous insulin infusion. Once euglycaemic and in steady state diuresis, patients received an intravenous infusion of either placebo (50mls of 0.9%NaCl) or atrial natriuretic peptide (0.025Ug/kg/min in 50mls of 0.9% NaCl) for 60mins. Results were analysed by ANOVA. **Results:** Baseline parameters were similar on both study days. Plasma levels of ANP increased significantly during the ANP infusion, but remained unchanged during the placebo limb, ($P < 0.001$). **Urinary albumin-creatinine ratio (ACR) increased significantly during ANP infusion when compared to placebo** (63.3 ± 42.3 to 869.1 ± 805.2 V 77.39 ± 72.3 to 78.42 ± 73.3 mg/g creat, $P = < 0.0001$) as did urine volume (Ur vol) (13.72 ± 2.79 to 28.11 ± 11.8 V 10.97 ± 3.72 to 14.08 ± 3.2 ml/min $P < 0.001$) and urinary sodium (UrNa) (16.14 ± 6.12 to 44.28 ± 29.84 V 20.28 ± 9.1 to 17.57 ± 8.1 mmol/L, $P < 0.001$). No changes in ANP, ACR, UrNa or Ur Vol were seen during placebo infusion. **Conclusion:** Intravenous infusion of atrial natriuretic peptide induces an increase in ACR in microalbuminuric patients with type 2 diabetes mellitus.

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Nephropathy: Markers

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IMPAIRED RESPONSE OF ALDOSTERONE TO ACTH INDEPENDENTLY PREDICTS END-STAGE RENAL DISEASE IN TYPE 2 DIABETES.

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Background and Aims: We hypothesized that an impairment of aldosterone response to ACTH could predict a group of type 2 diabetic patients who will rapidly reach end-stage renal disease (ESRD). To test our hypothesis, we assessed the association between the responsiveness of plasma aldosterone to ACTH at a much earlier stage before renal failure and the risk of ESRD. **Materials and Methods:** The subjects studied were 78 type 2 diabetic patients (46 men and 32 women, 54 ± 1 years) who were initially admitted to our hospital between April 1986 and December 1989. We excluded patients with hypoalbuminemia (serum albumin < 3 g/l) or renal failure (serum creatinine > 110 μ mol/l). The observation period was defined as the number of days from the date of ACTH to the date of introduced hemodialysis or 31 March 2000. ACTH injection was started after overnight recumbency. A blood sample for assays of plasma corticosteroids was obtained before and 30 and 60 minutes after the injection. Each value of (aldosterone AUC above baseline)/15 was treated as continuous variable in the statistical analysis (ranging from 0 to 67.1). Renal survival curve was estimated using the Kaplan-Meier product-limit method and compared by the log-rank test. The effects of prognostic factors on the outcome were evaluated by the Cox proportional-hazards model. **Results:** Of the 78 patients, 17 (21.8 %) were introduced to hemodialysis during the follow-up period (10.4 ± 0.4 years). When parameters selected in the univariate analysis were included in a stepwise Cox regression model, (aldosterone AUC above baseline)/15 and proteinuria were identified as the independent predictive factors of renal survival (relative risk=0.906, 95% CI; 0.849-0.966 and relative risk=5.165, 95% CI; 1.275-20.935, respectively). **Conclusions:** The present study for the first time indicates that an impairment of aldosterone response to ACTH is an independent and powerful predictor of ESRD in type 2 diabetes without azotemia.

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ROLE OF THE INTERCELLULAR ADHESION MOLECULE - 1 (ICAM - 1, CD 54) IN THE COURSE OF THE DIABETIC NEPHROPATHY IN TYPE 1 DIABETES MELLITUS (DM)

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Background and Aims: Cell adhesion molecules (CAMs) have been demonstrated to be increased in diabetes mellitus and implicated in the development of micro- and macrovascular complications. Intercellular adhesion molecule - 1 (ICAM - 1, CD 54) is expressed by different cell types. The aim of the study was to evaluate the clinical significance of the ICAM - 1 (CD 54) expression on leucocytes (lymphocytes and granulocytes) in Type 1 Diabetes and diabetic nephropathy (DN).

Materials and Methods: the cohort included 41 patients (16 males & 25 females, age mediana 26,4 years) with Type 1 Diabetes and DN. Persons with other autoimmune, inflammatory, oncological and hematological diseases were not included into the study . Healthy controls (n = 17) fitted the study inclusion criteria. The CD 54 expression on the surface of the blood lymphocytes and granulocytes was examined by flow cytometry using FACScan (Becton - Dickinson) . The data achieved presented on the Table 1.

Results & Conclusion

Table 1. ICAM - 1 (CD 54) Expression on Lymphocytes and Granulocytes in Patients With Type 1 Diabetes and Diabetic Nephropathy.

Characteristics	Group I Normoalbuminuria	Group II Microalbuminuria	Group III Proteinuria	Healthy Controls
N of patients	17	8	16	17
HbA1c (%)	8.0 ± 0.3	8.3 ± 0.2	8.5 ± 0.6	5.1 ± 0.3
CD 54 ^{pos}	12.18 ± 2.91	20.84 ± 7.18	20.41 ± 2.36^a	12.41 ± 3.02
Lymphocytes (%)				
CD 54 ^{pos}	11.40 ± 3.35	14.92 ± 6.67	36.91 ± 19^{ab}	8.33 ± 3.01
Granulocytes (%)				

A - versus controls ($p < 0.05$); b - versus controls & normoalbuminuria ($p < 0.005$) & microalbuminuria ($p < 0.05$)

We observe the rising CD 54 expression tendency in microalbuminuric and especially in proteinuric patients, reflecting the increased activative and adhesive ability of leucocytes on these stages. There needs further investigation of this mechanism, its correlation with metabolic control and DN prognosis .

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SERUM CYSTATIN C IN TYPE 2 DIABETES : A RELIABLE MARKER FOR EARLY GLOMERULAR DYSFUNCTION

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Background and Aims : Human cystatin C (Cys C), previously named γ trace has been proposed as a suitable marker for glomerular filtration rate (GFR). This study was conducted to determine the potential of Cys C as compared to serum-creatinine (S.C.) as a screening test for glomerular function in type 2 diabetics with variable degrees of renal affection. **Subjects and Methods :** This study included eighty subjects : forty six males and thirty four females with age range 17-64 years ($M \pm SD$ 40.86 \pm 11.50 years) were enrolled and subjected to complete clinical, urological, sonographic and histological examination. They were classified into 4 groups. Group I : 20 control subject, group II : 20 diabetics with over renal failure undergoing conservative treatment, group III : 20 diabetics with end stage renal disease undergoing regular hemodialysis and group IV : including 20 diabetic patients, 10 of them with microalbuminuria forming group IV-A and 10 patients with macroalbuminuria forming group IV-B. **Results :** Both S.C. and Cys C, were equally significantly elevated among group II and group III with P values <0.01 for both parameters in both groups. Both S.C. and Cys C showed an equal negative correlation to either of creatinine clearance (Cr cl) and GFR in the same groups II, III ($P < 0.01$ for both) Cys C was significantly higher in diabetic patients with early nephropathy (group IV) compared to the control group I ($P < 0.05$), with non significant elevation of S.C. ($P > 0.05$). In patients with microalbuminuria group IV-A S.C. did not correlate to either of Cr cl or GFR ($P > 0.05$ for both) while Cys C showed significant correlation towards the same items with P value <0.05. In patients with microalbuminuria sensitivity of S.C. in reflecting GFR was 95%, 100% and 60% in groups II, III and IV versus sensitivity of 100%, 100% and 80% for Cys C in the same groups in order. Specificity of S.C. and Cys C was almost equal in all groups. **Conclusion :** This study confirmed the reliability of Cys C as a marker for GFR in diabetic patients with different stages of nephropathy. It is as good as S.C. in follow up of overt renal failure patients but Cys C is a better marker than S.C. in early detection of GFR changes in diabetic patients.

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ELEVATED TRIGLYCERID LEVELS ARE A SIGNIFICANT RISK FACTOR FOR EARLY DEVELOPMENT OF DIABETIC NEPHROPATHY IN TYPE 2 DIABETIC PATIENTS

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Background and Aims: Age, duration of diabetes, glycemic control and hypertension are established risk factors for the development of microvascular complications in type 2 diabetic patients. Complication free duration of diabetes is variable in different patients, the protecting or promoting mechanisms are not completely elucidated. Animal and in vitro data suggest that dyslipidemia plays a major role in the initiation and progression of nephropathy. The aim of the following study was to identify type 2 diabetic patients with early onset of nephropathy and to analyse the contributing risk factors.

Materials and Methods: A total of 171 patients with type 2 diabetes mellitus participated in this cross-sectional and longitudinal analysis (Age:48 \pm 10 years, m/f: 97/74, 51% had a co-existence of hypertension). 44% of patients got nephropathy after a duration of diabetes of 13 \pm 9 years. The investigated risk factors were: Age, glycemic control, cholesterol, triglycerides, ACE-polymorphism, gender and co-existence of hypertension at diagnosis of diabetes.

Results: The univariate models showed a statistically significant effect of age ($p < 0.0001$) and triglycerides ($p < 0.0005$) at diagnosis on early development of nephropathy. Comparing patients with triglycerides of 250 mg/dl to patients with triglycerides of 150 mg/dl, relative risk was 1.4. The multiple regression model showed an independent significant effect of age ($p < 0.0001$) and triglycerides at diagnosis ($p < 0.005$) and also gender ($p < 0.03$) on the incidence of nephropathy. Glycemic control, cholesterol, a coexistence of hypertension at diagnosis and ACE-polymorphism did not have any influence on early onset of nephropathy.

Conclusions: Our results show, that elevated triglycerid-levels, established markers for insulin resistance, predict an increased risk for nephropathy. The early and aggressive treatment of hypertriglyceridemia could probably decrease the risk for the incidence of renal damage.

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Impact of Sodium Intake on 24hr-Blood Pressure and Albuminuria in Type 2 Diabetic Patients.

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Background and Aims: The effect of sodium intake on 24hr-blood pressure, albumin excretion rate, insulin sensitivity and kidney haemodynamics was examined in 20 Type 2 diabetic patients with (DM2+) and 21 without microalbuminuria (DM2-) well matched for metabolic control, BMI and blood pressure.

Materials and Methods: The patients underwent two consecutive 7 days periods on a high- (250 mEq) or low-sodium (20 mEq) diet. Body weight, 24hr-blood pressure, and albuminuria were measured in the last 3 days of each diet period. Insulin-sensitivity (euglycaemic insulin clamp; 2 mU/kg/min) and kidney haemodynamics were measured at the end of high-sodium diet in 9 DM2+ and 7 DM2-. Intraglomerular pressure was calculated from glomerular filtration rate, renal plasma flow, plasma protein concentration and pressure-natriuresis relationships.

Results: In DM2+, switching from a low- to high-sodium intake resulted in an increase in 24hr-blood pressure (+7.4 \pm 4.7 mmHg; $p < 0.001$), while no change occurred in DM2- (+1.3 \pm 3.4). After high-sodium diet, DM2+ gained more weight (+1.9 \pm 0.4 vs +0.6 \pm 0.3 Kg; $p < 0.05$). Albuminuria did not change in DM2- (from 8mcg/min[1-18][median and range] on low-sodium to 11 [3-27] on high-sodium diet), but increased in DM2+ from 80[31-183] to 101[27-965]; $p < 0.0$. Insulin sensitivity was lower in DM2+ (5.0 \pm 0.5 vs 7.2 \pm 0.7 mg/kg/min; $p < 0.01$). DM2+ also had greater intraglomerular pressure than DM2- (48 \pm 3 mmHg vs 37 \pm 1; $p < 0.01$). Urinary albumin excretion ($r = 0.52$; $p < 0.05$) and insulin-sensitivity ($r = -0.59$; $p < 0.01$) were correlated with intraglomerular pressure, but not with systemic blood pressure.

Conclusions: High salt intake in DM2+ caused an increase in 24hr-blood pressure and albuminuria. These responses were associated with insulin resistance and increased intraglomerular pressure. Insulin resistance may contribute to greater salt sensitivity, increased intraglomerular pressure and albuminuria. The results underscore the therapeutic importance of regulation of salt intake in preventing renal damage in Type 2 diabetic patients.

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RENAL HEMODYNAMIC IN PRECLINICAL NEPHROPATHY IN DIABETES TYPE 1 MEASURED BY COLOR-DOPPLER ULTRASOUND
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Doppler sonography has been used clinically to examine intrarenal hemodynamic abnormalities. Few reports describing the application of doppler sonography in patients with diabetes mellitus have been published. **Aim** of this study was to evaluate the possible correlation between resistive index (RI) of renal artery and intrarenal vessels and the presence of diabetic nephropathy in 40 patients affected by diabetes type 1. **Patients and methods** We divided the patients in two groups in relation to the urinary albumin excretion (UAE) values: ♦ group 1: 20 patients (6F 14M; age 36 \pm 6 yrs, duration of diabetes 18 \pm 5 yrs) with UAE <20 μ g/min ♦ group 2: 20 patients (8F 12M; age 33 \pm 5 yrs; duration of diabetes 22 \pm 6 yrs) with UAE \geq 20 and <200 μ g/min. All group 2 patients underwent ACE inhibitors therapy.

Ultrasound examination was performed by a triplex doppler apparatus (HDI ATL 3000). All measurements were performed by the same examiner who was unaware of the subject's characteristics. **Results** We observed that both intrarenal vessels RI and renal artery RI values were significantly higher in group 2 than those in group 1. In particular intrarenal vessels RI was 0.60 \pm 0.04 in group 1 and 0.64 \pm 0.04 in group 2 ($p = 0.003$) and renal artery RI was 0.64 \pm 0.04 in group 1 and 0.69 \pm 0.04 in group 2 ($p = 0.001$). We also analyzed the intima medial thickness of the common carotid arteries (IMT) and we found that it was higher in group 1 (0.76 \pm 0.1 mm) than in group 2 (0.69 \pm 0.1 mm) but this difference was not statistically significant. When we compared IMT and RI values we didn't find any correlation between them.

Conclusions These results demonstrate that: 1) type 1 diabetics with a preclinical nephropathy show a different intrarenal hemodynamic compared to those without renal complications; 2) no correlation exists between renal RI and carotid IMT, marker of macrovascular damage, in diabetes type 1.

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RENAL DUPLEX DOPPLER SONOGRAPHY IN PATIENTS WITH INSULIN DEPENDENT DIABETES COMPLICATED BY EARLY NEPHROPATHY

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Background and Aims: Diabetic nephropathy affects a subset of about 40% of patients with insulin-dependent diabetes mellitus (IDDM) and 30-50% of patients with non insulin dependent diabetes mellitus (NIDDM), after a period of 15-20 years. It is usually divided in 5 stages: the first 3 are characterized by renal hypertrophy and increased glomerular filtration surface area (I stage) followed by glomerular histological lesion (II stage) and early nephropathy with microalbuminuria (III stage). At these stages nephropathy is still reversible by medical treatment (ACE inhibitors) and good metabolic control. Aim of this study was to assess the usefulness of Duplex sonography with Doppler waveform analysis in the evaluation of early diabetic nephropathy, in order to detect patients at risk for irreversible renal disease. **Materials and Methods:** Fifteen patients (10 males, 5 females) aged 28-46 years, affected by IDDM were studied; 15 healthy subjects (7 males, 8 females) aged 20-45 years composed the control group. All of them underwent Duplex Doppler sonography of kidney; a scanner with 3.5 Mhz transducer (Toshiba 270 SSA) was used. All patients had renal function tests within normal range. Pulsatility index (P.I.) and Resistive index (R.I.) of Doppler waveform were obtained at the interlobar arteries; the average value of 3 bilateral measurements was taken. The same author without knowledge of the studied group (patient or control) did Doppler sonography. **Results:** Both indexes (P.I. and R.I.) showed higher values in patients with IDDM compared to controls: P.I. = 1.46 ± 0.30 vs. 1.07 ± 0.06 , $p < 0.05$; R.I. = 0.77 ± 0.09 vs. 0.60 ± 0.03 , $p < 0.05$. **Conclusions:** Even if our data have to be confirmed by further studies, they suggest that Duplex Doppler sonography may be useful complementary test in the evaluation of diabetic nephropathy, even in early stages.

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CLINICAL VARIABILITY IN URINARY ALBUMIN:CREATININE RATIOS

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The diagnosis of microalbuminuria is of major clinic importance in diabetes. Due to the variability of urinary albumin excretion an abnormal ACR needs to be confirmed. We assessed the variability of ACR in 3 early morning, first-voided urine samples in a hospital diabetes clinic setting. 228 patients, 139 men, 89 women, aged 58 (11-85 years) (median (range)), with a clinic ACR in the microalbuminuric range were studied. The median (range) of the 3 ACR levels was 2.8 (1.0 - 49.6) mg/mmol. The CV of the collections was 15 (0-117)%. The variability was similar between men and women (13 (0-111) vs 18 (0-117)%, respectively). Mean ACR values were divided below (LOW) and above (HIGH) 4.0 mg/mmol. The median (range) CV of the LOW group was similar to that of the HIGH group (12 (0-117) vs 19 (1-111)%, n.s.). In a second experiment the variability when only 2 ACR results were used was compared with the variability when 3 results were used. The median (range) of the CV for each set of 2 collections was 13 (0-107)% compared with 15 (0-117)% for 3 ACR values. Again the samples were divided below and above 4.0mg/mmol. In the LOW group the median (range) CV when only 2 ACRs were used was 9 (0-91)% compared with 12 (0-73)% when 3 results were used. For the HIGH group the results were 18 (0-106)% and 19 (1-111)% when 2 and 3 ACR results were used respectively. Gender had no effect whether 2 or 3 collections were used. In summary this large clinical survey has shown a lower variance in ACR levels in the microalbuminuric range compared to published series. Two ACR measurements in first-voided early morning urine samples provides the same information as three measurements.

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INSULIN SENSITIVITY AS A PREDICTOR OF TYPE 2 DIABETIC NEPHROPATHY: RESULTS FROM A CROSS-SECTIONAL STUDY

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Background and Aims: Impaired insulin sensitivity (IS) clusters with type 2 diabetes mellitus, hypertension, obesity, dyslipidemia and increased cardiovascular risk. In this cross-sectional pilot study we examined the relationship between IS and other putative risk factors and markers of diabetic nephropathy (age, BMI, diabetes duration [DUR], BP, HbA1c, AER, GFR, creatinine, total cholesterol [CH], LDL-CH and triglycerides [TG]).

Materials and Methods: IS was measured by hyperinsulinemic euglycemic clamp (insulin 80 mU/sq m/min) in 122 type 2 diabetic patients with normo- (n=31), micro- (n=56) and macroalbuminuria (n=35). GFR was measured by iohexol plasma clearance. Data were analysed by descriptive statistics and regression analysis. DUR, AER and TG were log-transformed.

Results: Age, BMI and HbA1c were similar in the three groups. Normoalbuminurics showed higher IS (M-value, mean 7.13, SD 2.89 mg/kg/min, $p < 0.05$) than micro- and macroalbuminuric patients (5.58, 2.19 and 4.75, 1.71, respectively). Macroalbuminuric patients showed highest BP in spite of treatment, highest plasma levels of TG, LDL-cholesterol and total CH as well as longest DUR ($p < 0.05$). In patients with increased lnAER, IS was not significantly related to GFR, HbA1c, total CH and TG.

Conclusions: A greater insulin resistance characterizes type 2 diabetic patients with abnormal AER regardless of diabetes duration, metabolic control and other concomitant risk factors. Despite the limitations of this cross-sectional design, these data suggest that insulin sensitivity might be an independent predictor of diabetic nephropathy and its cardiovascular complications.

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THE ROLE OF THE PROLYL ENDOPEPTIDASE IN DIABETIC NEPHROPATHY

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Background and Aims: Prolyl endopeptidase (PREP) is involved in degradation of angiotensin I (Ang I) and angiotensin II (Ang II) to angiotensin-(1-7). The process of cleaving of Ang I and Ang II to angiotensin-(1-7) by prolyl endopeptidase may be involved in the pathogenesis of diabetic nephropathy. The aim of the study was to find a genetic variant in the prolyl endopeptidase gene and examine its role in diabetic nephropathy.

Materials and Methods: We compared all cDNA sequences of the PREP gene from the available databases and found a frequent A/G polymorphism in the last exon of the gene, which changed valin to isoleucin. The polymorphism was confirmed by direct sequencing. A PCR-based RFLP protocol was designed with the SalI restriction enzyme. We collected two groups of type 2 diabetes patients: 294 patients with normoalbuminuria and 280 patients with nephropathy. Each patient was genotyped for the A/G polymorphism in the PREP gene and the frequency of genotypes was compared between the study groups.

Results: The genotype frequency was similar in the group with and without nephropathy. When we included in the normoalbuminuria group only those patients who were free of renal complication after at least 15 years of known diabetes duration we found a positive association between diabetic nephropathy and the A/G polymorphism in the PREP gene. The frequency of A allele carriers (AA and AG genotypes) was higher among patients with nephropathy than in patients with normoalbuminuria (30% vs 18%; $p = 0.039$).

Conclusions: The present study indicates that the A allele of the SalI polymorphism in the prolyl endopeptidase gene is a risk factor for nephropathy in patients with type 2 diabetes.

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SERUM ACTIVITY OF LYSOSOMAL ENZYMES AND URINARY GLYCOSAMINOGLYCAN EXCRETION IN DIABETIC NEPHROPATHY

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Background and Aims: The increased serum activity of lysosomal enzymes (LE) was found in diabetes mellitus (DM). It has been proposed that degradative action of circulating LE on endothelial and basement membrane glycoconjugates may be important in the pathogenesis of diabetic vascular complications. We studied the relationships between serum activity of LE, endothelial dysfunction and urinary glycosaminoglycan (GAG) in DM patients with and without nephropathy (DN). **Materials and Methods:** 46 type 1 DM patients (25 M, 21 F; age 30.3 ± 1.7 yrs) and 25 healthy controls were studied. 14 diabetes patients were normoalbuminuric (DN-), 32 micro- or macroalbuminuric (DN+). The activity of N-acetyl-glucosaminidase (NAG) and β -galactosidase in serum, the level of von Willebrand factor (vWF) antigen in plasma, the content and composition of GAG in urine were analysed.

Results: The high LE activity, vWF level and GAG-uria was found in DN+ patients:

	DN-	DN+	Controls
NAG (nmol \times h $^{-1}\times$ ml $^{-1}$)	875 \pm 59*	1267 \pm 67**	668 \pm 32
β -galactosidase (nmol \times h $^{-1}\times$ ml $^{-1}$)	1.35 \pm 0.16	1.73 \pm 0.23*	0.90 \pm 0.16
vWF (%)	109.3 \pm 5.9	139.9 \pm 4.5**	101.7 \pm 1.3
GAG (mg/mmol creatinine)	5.29 \pm 0.69*	6.22 \pm 0.55*	2.32 \pm 0.19

* - $p < 0.05$ v. s. control; ** - $p < 0.05$ v. s. control and DN- group; Mean \pm SEM

Both LE correlated positively with albuminuria ($p < 0.001$) and vWF ($p < 0.01$). Correlation between LE and GAG was significant ($p < 0.02$) in DN+ group only. The main urinary GAG in the control was chondroitin sulfate. Two DN- and 22 DN+ patients had increased heparan sulfate excretion and demonstrated the highest NAG and β -galactosidase activity (1486 ± 63 and 1.87 ± 0.19 nmol \times h $^{-1}\times$ ml $^{-1}$ respectively). **Conclusions:** High lysosomal glycohydrolase serum activity in complicated type 1 DM is associated with albuminuria, endothelial dysfunction and increased urinary GAG (mainly heparan sulfate) excretion, suggesting that LE may be involved in the pathogenesis of diabetic nephropathy.

PS 78

Nephropathy: Clinical

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COURSE OF NEPHROPATHY AND PROGNOSIS OF PROTEINURIC TYPE 2 DIABETIC PATIENTS OVER 3 DECADES (1970-2000)

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Background and Aims: Diabetic nephropathy (DN) is the most common cause of renal replacement therapy although treatment modalities have significantly improved in recent years. We investigated quality of treatment and course of nephropathy during the last 3 decades. **Patients and methods:** 70 type 2 diabetic patients with macroalbuminuria who were treated in the 1970s (n=22), 1980s (n=22) and 1990s (n=22) in Heidelberg. The following parameters were investigated: metabolic control (blood glucose HbA1c) blood pressure and lipid levels (cholesterol, triglycerides), loss of kidney function (creatinine clearance). Type 2 diabetics without DN treated during the same time period (n=20/24/23) served as controls. The mean duration of observation was 54 months. **Results:** Patients with DN showed a clear improvement in the 1980s and the 1990s compared to the 1970s with respect to metabolic control, blood pressure control and lipid levels. There were no significant differences between the patient group of the 1980s and the 1990s. 20% of patients with DN in the 1980s but 93% in the 1990s were treated with ACE inhibitors. Loss of kidney function was diminished in patients with DN during the observation periods: -6.4 ml/min/year in the 1970s, -4.4 and -3.0 ml/min/year in the 1980s and 1990s respectively. The corresponding figures of the controls were: -0.7/-0.3/-1.6 ml/min/year. There was no significant difference in loss of kidney function between patients with and without DN in the 1990s. Life prognosis improved markedly over the decades in patients with DN: Five-year actuarial survival was 35%, 75%, 100%, the corresponding figures in controls were 83%/95%/100%. Thus in the 1990s there was no difference between both groups. **Conclusion:** renal and life prognosis could be markedly improved during the last 3 decades as a result of an intensified metabolic and blood pressure treatment. The improvement of prognosis in the 1990s may be due to a general use of ACE inhibitors in proteinuric patients.

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Five-years incidence and determinants of renal insufficiency in type 2 diabetes: the Casale Monferrato Study.

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Background and Aims: Diabetic nephropathy is becoming the leading cause of end-stage renal disease. Few follow-up studies have been conducted in Caucasian populations so that the natural history of the disease is poorly known. We have previously identified a large population-based cohort of type 2 diabetes and classified them according to overnight albumin excretion rate (AER) in normoalbuminuric (n. 765), microalbuminuric (n. 488) and macroalbuminuric (n. 268). Aims of this study were to assess in a 5-yr follow-up the cumulative incidence and the determinants of renal insufficiency (plasma creatinine values > 177 μ mol/l).

Materials and Methods: Baseline examinations were centralized. The time period was calculated from the baseline study (1991-92) to the follow-up examination (1996-98). Censored observations were patients who died and those who moved from the area; for them, medical records (hospital discharges, computerized data of centralized laboratory) were examined. Independent predictors of progression to renal insufficiency were identified with logistic regression.

Results: The follow-up examination was performed in 1356 patients (86% of the cohort); out of the remaining, 109 were dead and their death certificates were analyzed, 109 could not be traced. Mean (SD) follow-up was 4.8 (1.8) yrs, with median 5.27, giving a total of 6503.7 person-years of observations; 72 new cases of chronic renal insufficiency were found (incidence /1,000 11.07, 95% CI 8.79-13.95). Rates were higher in macro- than in micro- and normoalbuminuric patients: 37.75/1,000 (27.47-51.88) vs 10.33/1,000 (6.86-15.45) and 3.36/1,000 (1.86-6.07). The RR of the upper vs the lower tertile of HbA1c was 1.85 (0.97-3.51). In logistic regression, after adjustment for age, sex and antidiabetic treatment, the following variables were independently associated with progression: microalbuminuria (RR 3.12, 1.48-6.55), macroalbuminuria (RR 11.00, 5.35-22.59); systolic blood pressure ≥ 179 vs ≤ 138 mmHg (RR 2.25, 1.03-4.89); fibrinogen ≥ 4.1 vs ≤ 2.3 g/l (RR 2.69, 1.24-5.85) and uric acid ≥ 381 vs ≤ 256 μ mol/l (RR 2.62, 1.16-5.88).

Conclusions: This population-based study shows that 5-yr cumulative incidence of renal insufficiency is relevant and that macroalbuminuria is the strongest predictor.

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The progression of glomerulopathy during 8 years in young patients with Type 1 diabetes and microalbuminuria.

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Background and Aims: Investigate prospectively the relationship between renal function and structural glomerular changes in young patients with Type1 diabetes and microalbuminuria.

Materials and Methods: An 8-years prospective study, 18 patients, age (mean) 20.1 yrs, duration of diabetes 11 yrs, HbA1c 10.1%, albumin excretion rate (AER) 45 ug/min, GFR 140 ml/min/1.73sqm, BP 125/81 mm Hg. Kidney biopsies were taken at baseline (B) and after 8 yrs (F). Glomerular ultra structural parameters were analysed with stereological methods.

Results: (mean and 95% CI). During the study mean HbA1c was 9.3 (8.7-10.0)%, and AER and BP did not change. GFR decreased from 143 (130-156) 125 (114-135) ml/min/1.73sqm ($p<0.05$). Glomerular volume (GV), matrix [Vv(matrix/glom)] and mesangial [Vv(mes/glom)] volume fraction, and basement membrane thickness (BMT) showed a highly significant increase during the 8 years. Mean 8-years HbA1c was associated with structural change (delta) during the study Vv(mes/glom), $r=0.56$, $p=0.02$, negatively with GFR-F, $r=-0.61$, $p=0.01$ and delta GFR, $r=-0.49$, $p<0.05$ (i.e. high HbA1c = large decline in GFR). Delta AER during the study was correlated to Vv(mat/glom)-F, $r=0.52$, $p<0.05$, BMT-F, $r=0.49$, $p<0.05$, GV-F, $r=0.62$, $p<0.01$ and delta GV, $r=0.58$, $p=0.01$. With AER-F as the dependent variable, mean 8-yrs HbA1c ($p<0.03$), Vv(mat/glom)-B ($p=0.004$) and BMT-B ($p<0.005$) contributed significantly. With GFR-F as the dependent variable, only mean 8-years HbA1c contributed significantly, $p<0.01$. For delta GFR mean 8-yrs HbA1c and GFR-B was of significance ($sqR=0.62$). However, when GFR-B was excluded from the analysis, smoking, mean HbA1c and Vv(mes/glom)-B contributed significantly to the model ($sqR=0.59$). With change during the study in Vv(mes/glom) as dependent variable and mean 8-yrs HbA1c, mean systolic BP and smoking during the study as the independent variables, only HbA1c contributed significantly, $p<0.01$.

Conclusions: Long term hyperglycaemia, smoking, the degree glomerulopathy at baseline, and previous hyperfiltration predict the kidney function after twenty years duration of diabetes.

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PROTECTIVE ROLE OF FEMALE SEX IN THE DEVELOPMENT OF NEPHROPATHY IN TYPE 2 DIABETICS WITH HYPERTENSION

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Background and Aims: The coexistence of arterial hypertension and diabetic nephropathy (DN) in type 2 diabetic patients is very common. In order to investigate the probable influence of sex in the development of diabetic nephropathy we studied 226 hypertensive type 2 diabetic patients of our diabetic center. **Materials and Methods:** DN was diagnosed by the measurement of the albumin/creatinine ratio in a morning urine sample (normal values: men <2.5 and women <3.5 mg/mmol). The characteristics of our study population were:

	Men with DN	Men without DN	Women with DN	Women without DN
Patients	41	54	33	98
HbA1c (%)	6.8 \pm 1.7	6.4 \pm 1.9	7.8 \pm 1.9	6.4 \pm 1.4
Duration of DM (yrs)	10.7 \pm 6.3	10.0 \pm 6.3	16.4 \pm 6.7	9.4 \pm 6.3
Age (yrs)	66.0 \pm 8.8	65.3 \pm 7.7	65.3 \pm 9.5	62.2 \pm 7.5

Results: There was a statistically significant difference in the percentage of development of DN between hypertensive men and women (43.1% versus 25.2%, $p=0.005$). Hypertensive women who developed DN had significantly higher values of HbA1c ($p=0.005$, $F=4.44$) and duration of diabetes ($p<0.001$, $F=10.05$) as compared to hypertensive women without DN and the men of both groups. It must be noticed that in the population without DN there was not any significant difference between men (22 out of 54) and women (54 out of 98) who were treated with ACE inhibitors for their hypertension. **Conclusions:** The development of DN in hypertensive type 2 diabetic patients is significantly more frequent in men than in women. For the development of DN in hypertensive diabetic women a more prolonged course of diabetes and a less sufficient glycaemic control seem to be required.

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DIABETIC NEPHROPATHY: VASCULAR DISEASE AND SURVIVAL

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Background and Aims: Diabetic nephropathy results in increased cardiovascular morbidity and mortality. One of the policies of our combined diabetic-renal clinic is aggressive management of cardiovascular risk factors. The aims of this study were to determine the prevalence of cardiovascular disease at referral to the clinic and to determine which factors predicted death.

Materials and Methods: A retrospective audit of patients with type 2 diabetes and nephropathy attending a combined clinic was performed. Survival curves were derived from Kaplan Meier technique and the importance of factors influencing death were assessed by Cox regression analysis.

Results: 126 patients were identified (50% female, mean age 60.2y, median serum creatinine 195umol/l, median albumin:creatinine ratio 293mg/mmol). 68.3% had a documented clinical history of vascular disease at referral. Parameters at referral: BP 165/87, HbA1c 8.2%, cholesterol 6.4mmol/l. The median number of anti-hypertensive drugs prescribed was 1, 41% of patients were prescribed ACE inhibitors, 14% statins and 46% aspirin. During follow up improvements were seen in blood pressure and cholesterol (BP 155/77, $p<0.005$; cholesterol 5mmol/l, $p<0.005$). No change was seen in glycaemic control. The median number of anti-hypertensive agents prescribed increased to 3, 74% were prescribed ACE inhibitors, 31% statins and 68% aspirin. Median patient survival (95%CI) from time of referral was 61 months (46.3,75.7); death rate was 10% per year. 73% of deaths were the consequence of a vascular event. Factors predicting death on multivariate analysis were low haemoglobin, high albuminuria, female sex and the lack of use of aspirin at referral.

Conclusions: Patients with type 2 diabetes and nephropathy have well established vascular disease at referral to a combined clinic. Despite improvements in risk factor profile while attending the combined clinic, survival was poor. These patients are high risk patients and should be offered aggressive cardiovascular protection early.

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DIFFERENT METABOLIC AND RENAL EFFECTS OF INSULIN LISPRO IN MICRO AND MACROALBUMINURIC TYPE 2 DIABETICS

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Background and Aims: Insulin Lispro (L), by limiting post prandial hyperglycemia, might reduce glucose-induced hyperfiltration, a possible determinant of diabetic nephropathy. In two separate occasions two weeks apart we compared post prandial metabolic and renal response to a standard meal (692 kcal, 54.2% carbohydrates, 17.4% proteins, 28.4% lipids) after the s.c. injection of 0.1 U/kg body weight of L or Regular (R) insulin in 21 (11 macro and 10 microalbuminuric) type 2 diabetes with serum creatinine <1.5 mg/dl.

Materials and Methods: Mean arterial pressure (MAP), blood glucose (BG), GFR and RPF (inulin and PAH renal clearances), and urinary albumin excretion were measured over 2 h before and 6 h after the meal. Filtration fraction (FF), renal vascular resistances (RVR) and albumin fractional clearances were calculated by standard formulas.

Results: In macroalbuminurics, BG levels were significantly lower ($p<0.05$) with L than with R from minutes 60 to 180. Percentual changes vs. basal with L and R at hours 2, 4, 6 were respectively: MAP: 0.8(5.9) vs -3.9(7.7), 6.1(11.5) vs -1.6(11) ($p<0.05$), 8.9(12.4) vs 2.5(9.9); GFR: -6.3(4.7) vs 5.8(5) ($p<0.05$), 0.7(5.1) vs 11(6.8) ($p<0.05$), 4.4(3.4) vs 11(8.1); RPF: -4.9(19.2) vs 5.9(21.6), -4.5(22.4) vs 9.0(19.3), -0.9(28.1) vs 13.1(36.1), FF: -0.01(0.05) vs -0.01(0.05), -0.01(0.06) vs -0.02(0.04), 0.03(0.07) vs -0.01(0.07); RVR: 10(7) vs -5.6(5), 18(10) vs -8(5) ($p<0.05$), 19(1) vs 0(11), albumin fractional clearance: 26(20) vs 42(31), 13(18) vs 44(34), 36(38) vs 117(74). Percentual changes in GFR ($r=-0.84$) and RPF ($r=-0.77$) were significantly correlated ($p<0.05$) with L plasma levels, but not with R and BG. All patients had suppressed c-peptide. Changes in microalbuminurics were similar, but not significant.

Conclusions: L prevents postprandial hyperglycemia, renal vasodilation and hyperfiltration, and tends to limit albuminuria more effectively in macro than in microalbuminuric type 2 diabetes. Mechanisms additional to improved metabolic control are likely involved in renal response to L.

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EFFECT OF PROTEIN RESTRICTION ON PROGNOSIS IN TYPE 1 DIABETIC PATIENTS WITH DIABETIC NEPHROPATHY

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Background and aims: Protein restriction improves survival and delays the progression to end-stage renal disease (ESRD) in non-diabetic nephropathies. The aims of the study were to determine the effect of protein restriction on the decline in GFR and the development of ESRD or death in diabetic nephropathy. **Material and methods:** We performed a 4-year prospective, randomised, controlled trial comparing the effect of a low-protein diet (0.6 g protein/kg/day) with a usual-protein diet. Eighty-two type 1 diabetic patients with progressive diabetic nephropathy (pre-study mean decline in GFR of 7.1 (5.8 to 8.5) ml/min/year) consecutively entered the study. Protein intake (24-hr urinary nitrogen excretion), GFR (^{51}Cr -EDTA), AER (ELISA), BP (random zero sphygmomanometer) and HbA_{1c} (HPLC) were measured. Data are means with 95% CI. **Results:** At baseline, patients in the two diet groups were comparable regarding protein intake, BP, GFR, AER, HbA_{1c}, lipids, previous cardiovascular disease (CVD), antihypertensive therapy and smoking habits. During follow-up the usual-protein diet group consumed 1.02 (0.95 to 1.10) g of protein/kg/day, as compared with 0.89 (0.83 to 0.95) in the low-protein diet group ($p=0.005$). The mean declines in GFR were 3.9 (2.7 to 5.2) ml/min/year in the usual-protein diet group and 3.8 (2.8 to 4.8) in the low-protein diet group (NS). Death or ESRD occurred in 27% of patients on usual-protein diet, as compared with 10% on low-protein diet (log-rank test; $p=0.042$). The relative risk of death or ESRD was 0.23 (0.07 to 0.72) for patients assigned to low-protein diet, after adjustment for the presence at baseline of CVD ($p=0.01$). BP, AER, HbA_{1c}, lipids, antihypertensive therapy and smoking habits were comparable in the two diet groups during follow-up. **Conclusion:** Moderate protein restriction improves prognosis in type 1 diabetic patients with progressive diabetic nephropathy.

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BENEFICIAL EFFECT OF ACEI AND PENTOXYPHYLLINE VERSUS ACEI IN HYPERTENSIVE, MICROALBUMINURIC TYPE II DIABETICS

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Background and Aims: In advanced diabetic nephropathy, combined treatment with ACE inhibitors (ACEI) and pentoxifylline seems to offer a higher renoprotection than ACE inhibitors alone. The aim of this study was to evaluate beneficial effect of pentoxifylline addition to ACE inhibitor treatment in type II diabetic patient with hypertension and persistent microalbuminuria (MCA).

Materials and Method: Fifty type II diabetic hypertensive patients with MCA (30-300 mg/24 hrs in two measurements) offer at least 6 months ACEI treatment were identified (serum creatinine >150 nmol/L or advanced heart insufficiency were exclusion criteria). Patients were randomized: group I ($n=25$; lisinopril) and group II ($n=25$; lisinopril plus 600 mg pentoxifylline). Lisinopril dose was individualized in both groups according to blood pressure. All patients completed 6 months of follow up. Blood pressure (BP), BMI, urinary albumin excretion (UAE) and HbA_{1c} were monitored. Results were analyzed with SPSS 9.01 ANOVA and t-tests were used.

Results: Both groups were initially similar in terms of sex, age (58.7 vs. 58.3 yrs), diabetes duration (12.7 vs. 12.9 yrs), BMI, BP (systolic 149 vs. 148 mmHg, diastolic 82 vs. 83 mmHg), HbA_{1c} (7.7 vs. 7.9%), cholesterol, creatinine and smoking habits. Initial UAE were also similar (228 vs. 208 mg/24 hrs). 9 months after randomization UAE had decreased to mean 148-mg/24 hours ($p<0.001$) in group I and to mean 128-mg/24 hrs. ($p<0.001$) in group II. The other parameters remained similar in both groups, particularly BP (systolic 147 vs. 146, diastolic 80 vs. 79 mmHg) and HbA_{1c} (7.6 vs. 7.8%).

Conclusion: In type II diabetic patients with hypertension and persistent MCA despite ACEI treatment, the addition of pentoxifylline was associated with a better decline in UAE. This effect was independent of BP and metabolic control.

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Beneficial Effect of Thiazolidine Derivatives On Urinary Albumin Excretion In Type 2 Diabetes

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Background and Aims: Recently effects of thiazolidine derivatives (TD) on nephropathy of diabetic animals have been reported. This study was aimed to investigate whether TD had a protective effect on diabetic nephropathy.

Materials and Methods: Subjects were 178 type 2 diabetic outpatients of Kainan hospital. They were treated with TD, alpha-glucosidase inhibitors (aGI), insulin or sulfonylureas (SU). Urinary albumin/creatinine ratio (ACR), HbA_{1c}, BMI, blood pressure were observed for three months. Because ACRs were not normally distributed, its natural logarithm (LnACR) was used for analysis. Data were shown as means \pm s.d. **Results:** 1. Patients characteristics were age 59.3 \pm 10.4 years old, diabetes duration 8.4 \pm 7.3 years, ACR 65.9 \pm 129.9 mg/gCr, HbA_{1c} 9.1 \pm 1.7%, BMI 23.3 \pm 3.4 kg/m² and systolic and diastolic blood pressure 136.0 \pm 18.1 and 75.1 \pm 11.0 mmHg. These indices of TD treated patients were not significantly different from those of aGI or SU treated patients. But some of these indices of insulin treated patients were significantly different from those of the others; HbA_{1c} was 10.1 \pm 2.2% ($p<0.05$ vs. the others), BMI 21.4 \pm 2.3 kg/m² ($p<0.05$ vs. the others). 2. Basal HbA_{1c} of TD, aGI, insulin and SU was 8.9 \pm 1.0, 8.8 \pm 1.5, 10.1 \pm 2.2, 8.8 \pm 1.9%, respectively. HbA_{1c} after each 3 months treatment was 8.2 \pm 1.2, 8.1 \pm 1.5, 7.8 \pm 1.5, 7.3 \pm 1.1%, respectively. Improvement of HbA_{1c} of TD and aGI was significantly lower than those of insulin and SU ($p<0.01$). 3. Basal LnACR of TD, aGI, insulin and SU was 3.48 \pm 0.92, 3.17 \pm 1.09, 3.49 \pm 1.50, 3.15 \pm 1.28%, respectively. LnACR after each treatment was 3.24 \pm 0.90, 2.98 \pm 1.09, 3.16 \pm 1.28 and 3.05 \pm 1.26, respectively. Significant decrease of LnACR was observed in TD and insulin treated patients ($p<0.05$), but not in aGI and SU treated patients. Decrement of LnACR by treatment was not significantly different from each other, which was thought to be due to a small number of patients of each group. 4. Blood pressure was not significantly different between before and after the each treatment. ACE inhibitors and angiotensin 2 receptor antagonists were used to treat hypertension in some patients, however, its frequency of usage was not significantly different between before and after each treatment. **Conclusions:** It was observed that TD had a beneficial effect to decrease ACR in type 2 diabetic patients in 3 months treatment, whereas it had a weaker effect to improve HbA_{1c} than SU and insulin. This observation suggests that TD is an expectable agent not only to control type 2 diabetes but also to treat their diabetic nephropathy.

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URINARY TRACT INFECTIONS (UTI) IN DIABETIC PEOPLE: THE POSSIBLE ROLE OF THE METABOLIC CONTROL.

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Background and Aims. To check the frequency and eventual risk factors for UTI in diabetic patients.

Materials and Methods. Between Jan 1997-Dec 2000, 395 diabetic patients (229 females, 166 males, 84 type 1, 311 type 2, 14-88 age years), attending the Diabetes Outpatient Clinic of the Pisa S. Chiara Hospital, together with 252 non-diabetic control subjects (146 females, 106 males) from an Ambulatory Cardiology Unit, were screened for significant UTI (presence of at least 10^5 CFU/ml in a culture of clean voided midstream urine). Identification of urinary isolates was performed by conventional methods and the *in vitro* susceptibility to antimicrobials was tested by the Kirby-Bauer method.

Results. The frequency of significant UTI was 13.9% (55/395) in all diabetics, 17.9% (41/229) in females, 8.4% (14/166) in males. In the control subjects, UTI frequency was 14.3% (36/252), 18.5% (27/146) in females, 8.4% (9/106) in males.

The UTI frequency was 13.5% (7/52) in type 1 diabetic females, 19.2% (34/177) in type 2 females, 6.2% (2/32) in type 1 males, 8.9% (12/134) in type 2 males. *E. Coli* was the causative organism of significant UTI in more than 55% of cases both for diabetic and non-diabetic subjects. No differences were observed as to the frequency of various isolates and their patterns of antimicrobial susceptibility between diabetic and control people.

When multivariate regression analysis (for age, duration and quality of metabolic control of diabetes, microalbuminuria, leukocytosis and glomerular filtration rate) was employed, the presence of higher HbA_{1c} levels resulted a significant risk factor for UTI occurrence only in type 2 diabetic females.

Conclusions. In our experience, in males the same prevalence of significant UTI occurs both in diabetic as well non-diabetic patients. Contrary to recent findings, the frequency of bacteriuria is also similar in diabetic and non-diabetic females. In type 2 females, the UTI prevalence is significantly influenced by the impairment of the long-term (HbA_{1c}) metabolic control.

PS 79 Hypertension

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PPAR γ ACTIVATION WITH FARGLITAZAR DECREASED BLOOD PRESSURE IN NORMAL AND SPONTANEOUSLY HYPERTENSIVE RATS
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Background and Aims: Elevated BP and impaired endothelial-dependent vasorelaxation are common findings in patients with insulin resistance, metabolic syndrome, and Type 2 diabetes. Pharmacological therapy that enhances the action of insulin may have beneficial effects on BP regulation. **Materials and Methods:** To evaluate clinically significant effects of farglitazar (F), a PPAR γ agonist, on heart rate (HR) and systolic (SBP) and diastolic BP (DBP), normotensive (NTR) male Sprague Dawley and spontaneously hypertensive rats (SHR) were instrumented for direct measurement of SBP, DBP and HR in the conscious state by radiotelemetry. The experimental design was as follows: (i) a baseline run-in period, (ii) 7-day dosing and (iii) 7-day recovery period. NTR ($n=6$ /group) were treated with vehicle (V), 1 or 3 mg/kg F twice daily by oral gavage and SHR ($n=8$ /group) received 5 mg/kg F twice daily. Data for each animal were evaluated by comparing the changes from the predose 12 h daytime (inactive period, InP) and nighttime (active period, AcP) averages with the equivalent averages for each treatment day. **Results:** There were decreases in SBP and DBP in both NTR and SHR within 24 h of the start of dosing. Maximum changes were evident by 72 h and maintained throughout dosing. The maximum % decreases from pretreatment averages in NTR were 4.4 and 9.4% for DBP and 3.4 and 5.4% for SBP in the 1 and 3 mg/kg groups, respectively. BP in V rats increased slightly over the treatment period by a maximum of 3.3% (DBP) and 3.9% (SBP) during the AcP. In the SHR, the maximum % decreases in the AcP were 20% (DBP) and 13% (SBP). Heart rate changes were variable and showed no clear treatment-related effects. In NTR and SHR, BP-lowering effects of F were more apparent during the AcP than the InP. **Conclusions:** These data suggest that PPAR γ activation is associated with BP lowering, possibly by interfering with endogenous pressor systems and enhancing responsiveness to or production of endogenous vasodilators.

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BORDERLINE GLUCOSE INTOLERANCE AND 24-HOUR BLOOD PRESSURE RHYTHM

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Background and Aims: 24-hour blood pressure rhythm, with at least 10% blood pressure fall at night, is impaired in diabetes mellitus. It has not been established whether the subjects with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) also show disturbed circadian blood pressure rhythm. **Materials and Methods:** The study subjects were 47 normotensive persons in whom WHO oral glucose tolerance test (75 g) was performed in previous 3 months. They were divided into three groups according to OGTT results: group 1 - 18 IGT subjects (mean age 48.2 \pm 7.7 yrs); group 2 - 14 IFG subjects (mean age 51.0 \pm 8.9 yrs); group 3 (controls) - 15 healthy subjects (mean age 47.5 \pm 10.4 yrs). Day/night blood pressure variation was examined using ABP-Monitor Mobil-O-Graph (I.E.M., Stolberg, D), validated according to the British Hypertension Society and US Association for the Advancement of Medical Instrumentation criteria. Recordings were made at 20 min intervals during daytime and 30 min intervals during nighttime. Daytime was defined as a period between 6.00 am and 10.00 pm; and nighttime - between 10.00 pm and 6.00 am. **Results:** Mean 24-hour systolic (SBP), diastolic (DBP), nighttime fall in systolic (Δ SBP) and diastolic (Δ DBP) blood pressure values are presented in the table.

group	SBP (mmHg)	DBP (mmHg)	Δ SBP (%)	Δ DBP (%)
IGT	123.1 \pm 8.2	72.6 \pm 5.8	12.7 \pm 4.0	13.3 \pm 6.9
IFG	126.8 \pm 7.7	73.9 \pm 7.0	15.4 \pm 6.2	7.3 \pm 3.2 *
controls	121.0 \pm 7.2	69.4 \pm 4.9	13.1 \pm 4.8	15.2 \pm 7.6

* $p<0.01$ vs IGT and controls

Conclusions: IFG subjects show blunted 24-hour blood pressure rhythm, which may suggest that even borderline glucose intolerance may have detrimental effect on the cardiovascular system. However, the findings of this study require confirmation in larger cohorts.

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HYPERINSULINEMIA, ARTERIAL RIGIDITY AND SYMPATHETIC ACTIVITY IN HYPERTENSIVE OBESE AND TYPE 2 DIABETIC PATIENTS.

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Background and aims: A poor cardiovascular prognosis has been attributed to an increase in arterial rigidity. An increase in sympathetic activity may be involved in hypertension. The aim of this study was to compare cardiovascular vagosympathetic activity in normotensive and mildly hypertensive obese and type 2 diabetic patients, and to investigate the relationship between pulse pressure (an index for arterial rigidity) and sympathetic activity in this population.

Materials and Methods: Seventy normotensive and 32 hypertensive obese patients, and 18 normotensive and 14 hypertensive type 2 diabetic patients were compared to 21 healthy subjects. Heart rate (HR) and blood pressure (BP) variations were studied by spectral analysis (Finapres).

Results: In the four groups, during a 6-min period of controlled breathing rate, the high frequency peak of HR variations (vagal activity) was significantly reduced ($p<0.001$) and the ratio mid/high frequency peak of HR variations (vagosympathetic balance) was significantly increased ($p<0.001$). The MF peak of systolic BP variations in the standing position (sympathetic activity) did not differ between the four groups of patients and controls. In the normotensive and hypertensive obese patients, the MF peak of BP variations correlated positively with insulinemia and insulin resistance index (HOMA) ($p<0.03$ to $p<0.006$). In the hypertensive obese and diabetic patients, this peak correlated significantly with pulse pressure ($p<0.05$ and $p<0.0001$, respectively).

Conclusions: These results strongly suggest that 1) cardiac vagal activity is reduced in hypertensive obese and type 2 diabetic patients like in normotensive patients; 2) vascular sympathetic activity may be increased by insulin resistance-induced hyperinsulinemia and the increase in sympathetic activity is an important factor involved in arterial rigidity and hypertension in this population.

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SERUM LEVELS OF FIBRINOGEN, PAI-1 AND PLASMINOGEN IN TYPE-2 DIABETIC SUBJECTS WITH CARDIOVASCULAR DISEASE AND HYPERTENSION

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Background and Aims: Abnormalities in the fibrinolytic system have been associated with an increased risk for cardiovascular disease (CVD) in diabetic and non-diabetic subjects. Aims of the present study were to investigate serum levels of Fibrinogen (F), Plasminogen Activator Inhibitor-1 (PAI-1) and Plasminogen (P) in Type-2 Diabetic subjects with either CVD or Hypertension (H) and to determine its association with CVD risk factors. **Materials and Methods:** We studied 65 (26 men, 39 women) Type-2 diabetic subjects randomly selected from the Outpatient Diabetic Unit at Thrasio General Hospital in Athens, Greece. They were divided in three groups according to the existence of CVD (in terms of coronary heart disease, stroke and peripheral vascular disease) and H (defined according to WHO criteria). Thirteen (5 men, 8 women) subjects without CVD or H served as the Control group (C). CVD+ group consisted of 30 (12 men, 18 women) subjects and H+ group consisted of 22 (9 men, 13 women) subjects. Venous blood samples were drawn after an overnight fast for various analysis included F, PAI-1, P, PT, APTT, HbA1c. **Results:** All groups were comparable regarding sex, age, WHR, DBP and HbA1c. BMI and SBP were significantly higher in either CVD+ or H+ groups than C group ($p=0.028$, $p=0.004$, $p=0.008$, $p=0.000$ respectively). Duration of diabetes was also significantly greater in CVD+ group than C group ($p=0.003$) or H+ group (0.016). Serum Fibrinogen levels were significantly increased in H+ group compared to C group ($H+427.95\pm132.21$ vs 340.61 ± 45.41 , $p=0.017$). Serum levels of P, PAI-1, PT and APTT did not differ among studied groups ($p>0.05$). When all subjects were considered, linear regression analysis revealed that F was strongly correlated with P and PT ($p=0.000$, $p=0.046$ respectively), PAI-1 with DBP and WHR ($p=0.013$, $p=0.036$ respectively) and P with F ($p=0.000$). **Conclusions:** 1. Serum levels of Fibrinogen are increased in Type-2 diabetic subjects with H as compared to those without H. 2. P and PAI-1 do not differ in Type-2 diabetic subjects with CVD or H as compared to those without CVD or H. 3. Serum Fibrinogen concentration was strongly correlated with P and PT. 4. Serum PAI-1 concentration was strongly correlated with diastolic blood pressure and WHR.

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Renoprotective effects of intensive antihypertensive treatment in type 2 diabetes - goals and compliance of patients

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Background and Aims: Different professional associations determined quite different cut-off values for tight control of blood pressure in diabetic patients - less than 150/90 mmHg, less than 130/85 mmHg, and recently diastolic pressure values even less than 80 mmHg. Is there some level of blood pressure control which may be considered 'too low or too risky' for renal function in this population? What we can do to improve the compliance of our patients with antihypertensive treatment modalities? **Materials and Methods:** Seventy (30 female/40 male, mean (SD) age of 58 (10.4) years) hypertensive type 2 diabetic patients were assigned to intensive antihypertensive treatment aimed at attaining the goal blood pressure of 130/85 mmHg or less. Among them 20 (28.57%) patients were non-complying with any antihypertensive treatment. Nephropathy progression parameters (average annual increment of microalbuminuria and deterioration of glomerular filtration rate) were followed-up for a period of 5 years.

Results: In patients non-complying with antihypertensive treatment microalbuminuria (MA) progressed annually on average of $14.91 \pm 4.95\%$ while glomerular filtration rate (GFR) deteriorated by -7.99 ± 2.03 ml/min/year. In 23 (32.86%) patients blood pressure values of less than 150/90 mmHg were achieved. That was enough to slow down MA progression to $4.77 \pm 9.90\%$ per year ($p < 0.001$) and GFR deterioration to -3.51 ± 3.56 ml/min/year ($p < 0.001$). In 9 (12.86%) patients with blood pressure levels of less than 130/85 mmHg average annual increment of MA was reduced to $4.57 \pm 7.90\%$ ($p < 0.001$) and GFR deterioration was even slower (-1.67 ± 6.00 ml/min/year, $p < 0.001$). These beneficial effects were visible but less pronounced in 18 (25.71%) patients with diastolic pressure values less than 75 mmHg (MA increment: $8.32 \pm 9.59\%$ per year, $p < 0.05$; GFR: -4.50 ± 5.63 ml/min/year, $p < 0.05$).

Conclusions: Despite different levels of blood pressure control achieved, there was a significantly slower increment of UAE and significantly slower decline of GFR in all treatment groups in comparison with the group of patients non-complying with any antihypertensive treatment. There was no statistically significant difference between the three groups of patients complying with the treatment. Knowing this we have serious ethical burden of non-complying patients. Informing them of such results can be one of the modalities in increasing their compliance with the therapy.

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Prevalence of hypertension and associated risk factors in diabetes

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Aims: This study is to evaluate the prevalence of hypertension among diabetics of eastern Algeria and to compare with hypertensive patients without diabetes, the prevalence of associated risk factors and diabetes complications. **Material and Method:** this study comprised hospitalised patients and who underwent medical control during 1994. A population of 567 diabetics (type 1: 225, type 2: 342) and a control group of 60 hypertensive non diabetics patients has been divided according to sex, age, duration of diabetes and hypertension ($140/90$ mmHg). Weight, height, hypercholesterolemia (> 260 mg/dl), hypertriglyceridemia (> 150 mg/dl), hyperuricemia (> 50 mg/dl), tabagism, family antecedents of diabetes and/or hypertension were recorded for all patients. The complications checked in the study were: coronary heart disease (CHD), strokes, arteriopathy, retinopathy and nephropathy. Statistical test: Chi 2. **Results:** The prevalence of hypertension was 31% in diabetic population. It has occurred three times more frequently in type 2: 27% than in type 1: 8% ($p < 10^{-8}$). Hypertension predominates in male (76%) with type 1 after forty. It occurs later, after 10 years of evolution of the diabetes and it's frequently associated during this period with a nephropathy (71%). Hypertension in type 2 is more frequent in women (66%) after fifty. It occurs before (36%), at the time (13%) or after (57%) the diabetes. The interval between the two diseases is inferior to five years in 44% of the patients. Complications are more frequent and precocious in hypertensive diabetics, than in the normotensive diabetics and in the control group. In the group of hypertensive type 2 diabetics, these complications are occurring in the following order of frequency: neuropathy (50%), CHD (40%), retinopathy (35%), strokes (33%). In hypertensive type 1 diabetics: neuropathy (65%), retinopathy (59%), arteriopathy (29%), CHD (24%), nephropathy and strokes (18%). Risk factors are more frequently associated to hypertension and cardio-vascular complications in hypertensive diabetics compared to normotensives as well as to the control group. BMI (> 26): 26%, 20%, 15%, ($p < 0.04$) - hypercholesterolemia: 43%, 24%, 26% - hypertriglyceridemia: 44%, 28%, 36% ($p < 0.001$) - tabagism: 38%, 29%, 22%. The frequency of a family antecedents of hypertension is higher in hypertensive diabetics in comparison to patients without hypertension. The treatment of the hypertension has been more of a monotherapy than a bitherapy, with ACE inhibitors and calcium inhibitors used first.

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HYPERTENSION IN TYPE 1 DIABETES - A POPULATION-BASED FOLLOW-UP STUDY

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Background and Aims: To determine the incidence of hypertension and its associated factors in type 1 diabetes within a geographically defined population.

Materials and Methods: The original records of all 1132 patients with onset of type 1 diabetes below age 40 diagnosed in the Erfurt district from 1966 to 1988 were analysed up to the end of 1990.

Results: Hypertension was diagnosed after 10 ± 7 years of diabetes at 35 ± 10 years of age. The cumulative incidence of hypertension (life table analysis) amounted to 62% after 25 years of diabetes and 78% by age 55, was higher in men, and showed a steeper increase after 20 years of diabetes in patients up to age 10 at diabetes onset. Patients with development of hypertension ($n=211$) had a significantly ($p < 0.05$) higher BMI (23.7 vs 22.6 kg/m²) at diabetes onset due to a lower height (170 vs 172 cm at the end of follow-up) and were born 2 years earlier (1949 vs 1951) compared to patients without hypertension matched for sex, age and duration of diabetes at the end of follow-up ($n=315$). They needed more insulin despite similar blood glucose and had higher systolic (132 vs 126 mmHg) and diastolic (85 vs 80 mmHg) blood pressure at diabetes onset which remained higher during the next 15 years. They suffered more often from myocardial infarction (6 vs 0.3%), stroke (3 vs 1%), claudication (13 vs 4%), and major foot amputation (3 vs 1%), developed more often proteinuria (30 vs 15%), chronic (10 vs 2%) and end-stage renal failure (2 vs 0%), background (58 vs 51%) and proliferative retinopathy (9 vs 2%) as well as blindness (2 vs 0%), and died with higher frequency (13 vs 4%) during the follow-up period.

Conclusions: The cluster described above resembles the metabolic syndrome. A lower height with birth shortly after World War II is in agreement with the small baby hypothesis of fetal malnutrition as one possible cause of this syndrome. Thus a subgroup of type 1 diabetes is at especially high risk for long-term complications and early death presumably due to a concurrent insulin resistance syndrome.

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Blood Pressure Distribution in Diabetes-Does it differ in Type 1 and Type 2 Diabetes?

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Background and Aims: Hypertension is more common in people with diabetes but may differ between type 1 and type 2 diabetes. There is scant evidence on whether the time course and natural history of hypertension, differ markedly between Type 1 and Type 2 diabetes. We evaluated the distribution of Systolic Blood Pressure (SBP) by age, in a population with Type 1 and Type 2 diabetes, in comparison to a population without diabetes.

Materials and Methods: Data from patients aged 20 to 80 years, comprising 1271 with Type 1 and 4442 with Type 2 diabetes, respectively, were analysed from the district diabetes register. The SBP distribution by age in these two groups was compared with that obtained from 5497 non-diabetic control subjects of similar age (UK Health and Lifestyle Survey). Statistical comparison was by analysis of variance using SPSS.

Results: All results are Mean+Standard Deviation given in non-diabetics, Type 1 and type 2 diabetes, respectively. Age in years, 43+15, 49+15 and 61+11 ($p < 0.0001$). Body mass index, 25+10, 27+7 and 29+8 ($p < 0.0001$). SBP in mmHg, 131+18, 142+23 and 151+23 ($p < 0.0001$). Diastolic blood pressure in mmHg, 81+12, 74+11 and 78+11 ($p < 0.0001$). The mean SBP after correction for difference in age, in mmHg, 134, 145 and 152 ($p < 0.0001$).

Conclusions: There were highly significant differences in blood pressure between patients with Type 1 and Type 2 diabetes, even accounting for age and both groups showed elevated SBP in comparison to normals. This suggests a potential link between diabetes and hypertension via the metabolic syndrome, most marked in Type 2 diabetes.

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Ambulatory pulse pressure is associated with micro- and macrovascular complications in type 2 diabetes

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Background and aims: In non-diabetic subjects pulse pressure is an independent predictor of cardiovascular disease and microalbuminuria. We investigated the association between retinopathy, nephropathy, macrovascular disease and pulse pressure in a group of type 2 diabetic patients.

Materials and Methods: In 80 type 2 diabetic patients we performed 24-h ambulatory BP measurement and fundus photographs (graded independently by two experienced graders according to ETDRS criteria). Urinary albumin excretion was evaluated by three urinary albumin/creatinine ratios. Presence or absence of macrovascular disease was assessed by an independent physician.

Results: 49 patients had no detectable retinal changes (grade 1), 13 had grade 2 retinopathy, and 18 had more advanced retinopathy (grades 3-6). Compared to patients without retinopathy (grade 1), patients with grades 2 and 3-6 had higher ambulatory pulse pressure: Day pulse pressure 57 ± 11 , 64 ± 11 , and 63 ± 15 mmHg, $p=NS$, and night pulse pressure 55 ± 10 , 64 ± 10 , and 61 ± 15 mmHg, $p<0.05$ (grade 1, 2, and 3-6 respectively). Comparing nephropathy groups (45 normo-, 19 micro-, and 15 macroalbuminuric patients) results were similar: Day pulse pressure 57 ± 9 , 59 ± 11 , and 70 ± 16 mmHg, $p<0.001$, and night pulse pressure 54 ± 9 , 57 ± 10 , and 70 ± 15 mmHg, $p<0.001$. Likewise, compared to patients without macrovascular disease ($n=55$), patients with this complication ($n=25$) had higher ambulatory pulse pressure values: Day pulse pressure 58 ± 12 vs. 63 ± 12 , $p=0.07$ and night PP 57 ± 12 vs. 63 ± 11 mmHg, $p<0.05$.

Conclusions: Micro- and macrovascular complications are associated with an increased pulse pressure in type 2 diabetes. This haemodynamic abnormality might contribute to the development of diabetic complications.

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AMBULATORY BLOOD PRESSURE PROFILES: A NOVEL MEANS OF IDENTIFYING UNCONTROLLED HYPERTENSION

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Background and Aims: In-office blood pressure (BP) measurement is known to under detect clinically significant HTN. We report a novel means of representation of data from ambulatory blood pressure monitoring. **Materials and Methods:** An Omron® IC BP monitor was used over a period of 2 weeks to automatically record systolic and diastolic BP with time and date. Ambulatory blood pressure profiles (ABPP) were created by collapsing all data into a single day and using a Tukey smoothing algorithm to produce continuous curves representing the 90th, 75th, 50th, 25th and 10th percentile systolic and diastolic BP. Data were compared by paired t-tests with 6 in-office BPs (sphygmomanometer and Omron® 905). **Results:** Forty-eight randomly selected patients (30M/18F) participated: mean age 64 ± 14 years, duration of type 2 DM 15.3 ± 9.6 years, duration of HTN 17.8 ± 13.7 years, and BMI $M=31.5 \pm 6.2$ kg/m², $F=35.3 \pm 9.2$ kg/m². Using the electronic monitors, subjects obtained 87.6 ± 14.6 BP measurements over 2 weeks. The mean ambulatory diastolic pressure (77.2 ± 8.7 mmHg) was significantly ($p<0.001$) higher than the mean in-office diastolic pressure (72.8 ± 10.6 mmHg). The ambulatory mean arterial pressure was also significantly higher (98.9 ± 8.4 vs. 95.5 ± 9.4 mmHg, $p<0.01$). No significant difference in mean systolic blood pressure (142.4 ± 14.2 vs. 141.1 ± 13.9 mmHg) was observed. However, when ABPPs were individually examined, 90% of the subjects had as many as 60% of their BP values elevated (systolic 10 - 60 mmHg, diastolic 5 - 20 mmHg). These elevations were not reflected in any of the in-office measures. **Conclusion:** Newer technologies may allow for a more accurate and sensitive measure of variation in BP throughout the day. In this study these variations revealed uncontrolled hypertension as well as potentially ineffective therapies in 90% of the subjects. This may suggest the need to reconsider current definitions of HTN and the means by which the efficacy of therapy is determined.

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ANTI-HYPERTENSIVE EFFECT OF FARGLITAZAR, A PPAR γ AGONIST, IN PATIENTS WITH TYPE 2 DIABETES AND HYPERTENSION

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Background and Aims: Farglitazar (formerly referred to as GI262570) is a novel, non-thiazolidinedione PPAR γ agonist derived from an L-tyrosine root with a high affinity for the human PPAR γ nuclear receptor, and low but possibly important activity for the related PPAR α receptor. Blood glucose lowering and blood lipid lowering activity have been demonstrated in people with Type 2 diabetes treated with farglitazar. Blood pressure (BP) lowering was noted in studies in normal subjects and in people with Type 2 diabetes. **Materials and Methods:** A 4-week randomised, double-blind, parallel-group study in 304 hypertensive Type 2 diabetic patients was therefore designed to define the dose-response relationship of the anti-hypertensive effects of farglitazar. Patients received either placebo or farglitazar 0.5, 1, 2, 5, or 10 mg daily. **Results:** After 4 weeks, farglitazar resulted in dose-dependent reductions in mean 24-hour ambulatory BP, which were statistically significant compared with placebo at the 5 mg [diastolic -4 (95%-CI -5, -2) mmHg $p<0.001$, systolic -5 (-7, -2) mmHg $p<0.001$] and 10 mg [diastolic -6 (-8, -5) mmHg $p<0.001$, systolic -8 (-11, -6) mmHg $p<0.001$] doses (baseline adjusted ANOVA). Farglitazar 10 mg resulted in a statistically and clinically significant lowering of mean seated trough diastolic [-6 (95%-CI -9, -3) mmHg $p<0.001$] and systolic [-6 (-10, -2) mmHg $p=0.004$] BP, with smaller non-significant decreases at lower doses. Farglitazar was well tolerated, with no clinically significant increase in heart rate. Dose-related oedema was seen (up to 13% at 10 mg after 4 weeks). The 10 mg dose is not being developed in Phase III. **Conclusions:** We conclude that the PPAR γ agonist farglitazar has moderate but potentially clinically useful anti-hypertensive properties to complement its glucose and lipid lowering effects.

PS 80

Nephropathy and Hypertension

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ANGIOTENSIN CONVERTING ENZYME INHIBITOR- AND ANGIOTENSIN II RECEPTOR ANTAGONIST-INDUCED SCAVENGING EFFECTS OF SUPEROXIDE MODULATE THE NEPHROPATHY IN TYPE 2 DIABETES. T. Inukai, K. Takamashi, K. Tayama, Y. Aso and Y. Takemura, Koshigaya, Japan
Background and Aims: Active oxygens produced from human body induce the tissue disorder and are a risk factor for developing diabetic complications. It has been accepted that angiotensin converting enzyme inhibitor (ACE-I) and angiotensin II receptor (type I) antagonist (AT₁R-A) have a potency of antioxidant effect, in addition to antihypertensive effect. We therefore investigated the relationship between the diabetic nephropathy and the potency of ACE-I and AT₁R-A on scavenging effect (SE) of superoxide (SO) in type 2 diabetes.
Materials and Methods: Studies were conducted using polymorphological leukocytes in type 2 diabetic patients (N=26) and healthy subjects (N=26). SE (U/10³ cells) of SO was measured by an electron spin resonance method. Among antioxidant drugs, we used ascorbic acid (AA) as a positive control, and did captopril (CP), temocapril (TP) (inactive type), temocaprilate (TPL) (active type) as ACE-Is, and then did RNH-6270 as a AT₁R-A. We measured urinary albumin excretion (UAE; mg/day) from daily stocked urine.
Results: SE after AA addition showed a marked response in a dose-dependent fashion. CP, TPL and RNH-6270 showed a dose-dependent SE response within the range of 10³ to 10⁷ mg/ml, respectively. TP alone did not exhibit any SE responses. The potency of Δ SE obtained from CP- and TPL-addition were relatively stronger than that of RNH-6270. Δ SE from all drugs used in diabetic patients were constitutionally similar to that in healthy subjects. Δ SE from TPL significantly reversely correlated with UAE in diabetic patients ($P < 0.05$). Δ SE from CP and RNH-6270 also tended to correlate reversely with UAE.
Conclusions: We demonstrated that both ACE-I and AT₁R-A definitely possessed scavenging effect of SO in leukocytes in type 2 diabetic patients and healthy subjects, and that those scavenging effects were possible to suppress the progression of diabetic nephropathy in diabetic patients.

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IRBESARTAN IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND MICROALBUMINURIA (IRMA II): DESIGN AND BASELINE CHARACTERISTICS
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Background and aims: The rationale for renin angiotensin system blockade in patients with hypertension (HT) and diabetes is well established. A Global Program for Irbesartan Mortality and Morbidity Evaluations (PRIME), was carried out to assess this hypothesis in type 2 diabetic patients. IRMA II study part of PRIME program was designed to determine whether Irbesartan (Ang II receptor antagonist), through its full Ang II blockade, can slow the progression from microalbuminuria to overt diabetic nephropathy in hypertensive type 2 diabetic patients with persistent microalbuminuria.
Material and methods: This was a multinational, randomised, double-blind study with 3 parallel groups followed for 2 years. Two doses of Irbesartan (150mg/daily, 300 mg/daily) were compared to standard antihypertensive treatment, so called 'placebo' aiming at equal control of system blood pressure in all groups. 611 patients with the following baseline characteristics (mean values (standard deviation)) were enrolled: Age 58 (8), male (68%), BMI (kg/m²) 30 (4), know duration of diabetes (years) 10 (8), patients on insulin treatment (35%), blood pressure (mmHg) 153 (14)/90 (9), know duration of hypertension (years) 7 (8), over night urinary albumin excretion rate (μ g/min) 64 (39), serum creatinine (μ mol/l) 94 (16), HbA_{1c} (%) 7.2 (1.7).
Results: Primary endpoint: Progression to overt nephropathy (albuminuria > 200 μ g/min and at least 30% increase from baseline).
 Secondary endpoints: Changes in urinary albumin excretion rate, creatinine clearance, von Willebrand factor, fibrinogen, factor 7, plasminogen activator inhibitor and lipid profile.
 The study ended in December 2000.
Conclusion: IRMA II will demonstrate the effect of Irbesartan on the progression of microalbuminuria to overt nephropathy in hypertensive patients with type 2 diabetes and persistent microalbuminuria. The trial data will be available in May 2001.

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MAINTAINED AUTOREGULATION OF GLOMERULAR FILTRATION RATE (GFR) DURING CANDESARTAN TREATMENT IN HYPERTENSIVE TYPE 2 DIABETIC PATIENTS.
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Background and Aims: Impaired autoregulation of GFR implies disturbances in the transmission of the systemic blood pressure (BP) into the glomerulus leading to capillary hypertension. The impact on renal autoregulation of different antihypertensive drugs in animals has been elucidated, while information in man is lacking. We therefore studied the effect of acute lowering of BP on GFR during candesartan treatment in Type 2 diabetic patients.
Materials and Methods: We performed a randomized double blind crossover study with candesartan cilexetil 16 mg o.d. and placebo in 17 hypertensive type 2 diabetic patients without nephropathy. Each treatment arm lasted 4 weeks. On the last day GFR (single shot [⁵¹Cr] EDTA plasma clearance technique for 4 hrs) was measured twice between 08.00 and 17.00: first without clonidine hereafter after intravenous injection of clonidine 75 μ g. BP (Takeda TM2420) was measured every 10 min and urinary albumin excretion rate (UAER) by ELISA during each GFR determination.
Results: Candesartan induced a mean (SE) reduction in mean arterial BP (MABP) of 6 (2) mmHg $p < 0.02$, and had a tendency to reduce UAER ($p = 0.07$), while GFR remained unchanged (95 vs 93 ml/min/1.73m²). Clonidine reduced MABP with 17 (2) vs 16 (1) mm Hg during placebo vs candesartan, respectively (NS). GFR diminished in average from 95 (3) to 92 (4) ml/min/1.73m² with placebo (NS), and from 93 (3) to 89 (4) ml/min/1.73m² during treatment with candesartan (NS). Mean difference (95% confidence interval) between changes in GFR between the examination with placebo and with candesartan were 0.1 (-5.5 to 5.8) ml/min/1.73m² (NS).
Conclusions: Our study suggest that candesartan reduce BP without adversely alter the preserved ability to autoregulate GFR in hypertensive type 2 diabetic patients without nephropathy.

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BENEFICIAL EFFECT OF CAPTOPRIL-DILTIAZEM VS CAPTOPRIL IN TYPE 2 DIABETIC, HYPERTENSIVE, MICROALBUMINURIC PATIENTS.
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Background and Aims: in advanced diabetic nephropathy, combined treatment with ACE inhibitors (ACEI) and nondihydropyridine calcium antagonists seems to offer a higher renoprotective effect than ACEI alone. In a preliminary report we suggested that in type 2 diabetic patients with hypertension and persistence of microalbuminuria (MCA) despite ACEI treatment, the addition of diltiazem was associated with a better evolution of urinary albumin excretion (UAE). We present the definitive results after a 2 year follow-up. **Material and Methods:** 36 type 2 diabetic, hypertensive patients, who remained microalbuminuric (30-300mg/24h) after at least one year of ACEI treatment were identified. Serum creatinine >150 μ mol/l or advanced heart insufficiency were exclusion criteria. Patients were randomised: group C (n=22; captopril) and C+DTZ (n=14; captopril and 120mg diltiazem). Captopril dose was individualized in both groups according to blood pressure. 28 patients completed two years of follow-up (group C, n=17; group C+DTZ, n=11). Results were analyzed with non-parametric tests. UAE was log transformed. **Results:** both groups were initially similar in terms of sex, age (61 years both groups), diabetes duration (14 vs 13 years), BMI (28.5 vs 29.0 kg/m²), BP (systolic: 148 vs 149 mmHg; diastolic 82 vs 83), HbA_{1c} (7.9 vs 8.4%), cholesterol, triglycerides, creatinine and smoking habits; initial UAE was also similar (128 vs 113 mg/24h). After two years, group C patients were receiving a higher dose of captopril (106 vs 73mg, $p < 0.05$). UAE only increased in group C (270mg/24h, $p < 0.05$) and was higher than in group C+DTZ (130mg/24h, $p < 0.05$). Seven patients in group C but only one in group C+DTZ progressed to macroalbuminuria. The other parameters remained similar in both groups, particularly BP (systolic: 146 vs 145, diastolic: 81 vs 78mmHg) and HbA_{1c}. **Conclusion:** in type 2 diabetic patients with hypertension and persistent MCA despite ACEI treatment, the addition of diltiazem was associated with a reduced progression to macroalbuminuria after two years of follow-up. This effect was independent of BP and metabolic control.

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Effects of losartan on urinary albumin excretion and endothelial vasomotor function in type 2 diabetic patients with microalbuminuria

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Background and Aims: Microalbuminuria is associated with dysfunction of the vascular endothelium in patients with diabetes mellitus. The aim of the present study was to investigate the effect of losartan, an angiotensin II receptor antagonist, on endothelial vasomotor function and urinary albumin excretion in type 2 diabetic patients with microalbuminuria.

Materials and Methods: Eighty diabetic patients were randomized to receiving either losartan 50 mg daily or placebo in a 6-month double-blind study. Inclusion criteria were HbA_{1c} <10%, urinary mean albumin excretion rate (MAER) 20-200 microgram/min and blood pressure <140/90 mmHg. Subjects on angiotensin converting enzyme inhibitor were excluded from the study. Endothelium-dependent and independent vasodilation were measured using high resolution vascular ultrasound.

Results: At baseline, the 2 groups were comparable in their blood pressure, MAER and endothelial function. Glycaemic control remained stable and blood pressure did not change significantly in either groups throughout the study. The MAER decreased in the losartan-treated group whereas an increase was observed in the placebo group. At 6 months, the losartan-treated group had significantly lower MAER than the placebo-treated group [54.5 (58.3) microgram/min vs 78.5 (100.5), $p < 0.05$; median (interquartile range)]. No significant differences were found in endothelium-dependent ($6.1 \pm 2.4\%$ vs $5.8 \pm 2.4\%$) or independent vasodilation ($13.0 \pm 4.1\%$ vs $13.0 \pm 3.1\%$).

Conclusions: Losartan has a urinary albumin lowering effect independent of its effect on blood pressure. It prevents deterioration of microalbuminuria in patients with type 2 diabetes mellitus without altering endothelial function.

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COMBINATION OF AT₁RB AND ACE-I HAS A SYNERGIC EFFECT ON THE REDUCTION OF MICROALBUMINURIA IN TYPE 2 DIABETIC PATIENTS

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Aims: Albuminuria is a sensitive predictor of microangiopathy in diabetic patients. We have already reported that Losartan (L), an angiotensin II receptor antagonist (AT₁RB), has a beneficial effect in the early stages of diabetic nephropathy similar to Enalapril (E), an angiotensin converting enzyme inhibitor (ACE-I). In this study, we have investigated the effect of combination of treatments with L and E on the prevention of the early stages of nephropathy in type 2 diabetic patients with hypertension. **Methods:** We randomly divided the 102 subjects into 5 groups; 1) subjects who received 100 mg of L alone, 2) 10 mg daily of E alone, and 3) 50 mg of L and 5 mg of E from the beginning of the therapy, 4) addition of 5 mg of E at 6 months after 50 mg of L, and 5) addition of 50 mg of L at 6 months after 5 mg of E. The changes in the mean blood pressure (mBP) and albumin excretion rate (AER) in each group were assessed before, 6 months and 12 months after the start of each protocol. **Results:** The blood pressure was controlled under 140/90 mmHg in all groups, and there were no significant differences in the reduction rate of mBP at 12 months. The degree of reduction in AER of group 3, 4 and 5 at 12 months was significantly greater than that of group 1 and 2 (* $p < 0.05$) among the groups (table). **Conclusions:** These results suggest that the combination therapy of ACE-I and AT₁RB has a synergic effect on the reduction of microalbuminuria in type 2 diabetic patients with hypertension.

Group	1 (n=25)	2 (n=19)	3 (n=18)	4 (n=17)	5 (n=23)
Δ mBP (mmHg)	-31.8	-40.2	-32.3	-37.3	-37.6
Δ AER (%)	-46.2	-42.1	-52.3*	-54.2*	-62.3*

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EFFECTS OF ENALAPRIL AND LOSARTAN ON GLOMERULAR ANIONIC CHARGE IN HYPERTENSIVE TYPE 2 DIABETIC PATIENTS

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Background and Aims: Animal studies have shown that, besides diminishing the glomerular pressure, Angiotensin-Converting enzyme (ACE) inhibitors might reduce urinary albumin excretion (UAE) by influencing glomerular charge selectivity through preservation of glycosaminoglycans. Effects of Angiotensin II (AII) antagonism on glomerular charge remain to be determined. The aim of this study was to compare the effects of AII antagonist, Losartan, and ACE inhibitor, Enalapril on UAE, urinary glycosaminoglycan excretion (Ugag) and red blood cell membrane anionic charge (RBCCh) which are the indirect markers of glomerular basement membrane anionic content, in hypertensive type 2 diabetic patients. **Materials and Methods:** Twenty four mild to moderately hypertensive type 2 diabetic patients were randomised into 2 groups. Twelve patients (Group E, M/F 2/10, 52.8±5.5 years) received enalapril maleate (5-40 mg/d) and 12 (Group L, M/F 4/8, 51.9±6.5 years) received Losartan potassium (50-100 mg/d). Following parameters were measured at baseline and after 6 months of treatment. UAE was measured by nephelometry. Ugag determined spectrophotometrically after addition of 1,9-dimethylmethylene blue and RBCCh by alcian blue binding test. Blood pressures were followed. **Results:** At the end of 6 month period, systolic and diastolic blood pressures were significantly reduced in both treatment groups ($p < 0.05$). Pre- and post-treatment UAE levels were 83.5±19.5 mg/d vs 25.9±10.8 mg/d ($p < 0.05$) for group E, 80.1±19.2 mg/d vs 26.2±5.2 mg/d ($p < 0.05$) for group L. Ugag excretion was 50.2±19.1 mg/d vs 25.1±17.4 mg/d ($p < 0.05$) for group E and 58.2±22.6 mg/d vs 28.5±18 mg/d ($p < 0.05$) for group L. RBCCh were 166.7±72.4 ng/alcan blue/10⁻⁶ RBC vs 433.2±177 ng/alcan blue/10⁻⁶ RBC ($p < 0.01$) for group E and 163.5±74.3 ng/alcan blue/10⁻⁶ RBC vs 368.4±141.8 ng/alcan blue/10⁻⁶ RBC for group L. Pre- and post treatment respectively, UAE was negatively correlated with RBCCh ($r = -0.41, p < 0.05$) and positively correlated with Ugag ($r = 0.41, p < 0.05$). RBCCh was found to be negatively correlated with Ugag in all study group ($r = -0.44, p < 0.02$). **Conclusion:** Enalapril and Losartan treatment were equally effective in reducing UAE as well as Ugag excretion and preserving RBCCh in hypertensive type 2 diabetic patients. ACE inhibition and AII antagonism might have additive effects on preserving glomerular basement membrane anionic content in diabetic patients.

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Dual blockade of the renin-angiotensin system in type 1 diabetic patients with diabetic nephropathy resistant to antihypertensive treatment

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Background and Aims: Albuminuria and hypertension are strong predictors of poor renal and cardiovascular outcome in diabetic patients. Approximately 30 % of type 1 patients with diabetic nephropathy (DN) have albuminuria >1g/day and blood pressure >135 and/or >85 mm Hg ("the resistant patient") despite antihypertensive combination therapy including diuretics and the maximally recommended dose of ACE inhibitor (ACEi). We tested the effect of dual blockade of the renin-angiotensin system (RAS) in these patients.

Materials and Methods: We performed a randomised double blind crossover study with 2 months treatment with Irbesartan 300 mg o.d. and placebo added on top of previous antihypertensive treatment. We included 21 type 1 diabetic patients with DN resistant to conventional treatment as defined above. All received ACEi treatment and diuretics, in addition 10 patients received a calcium channel antagonist and 3 patients a β-blocker. At the end of each treatment period albuminuria, 24-hour blood pressure and glomerular filtration rate (GFR) were measured.

Results: Addition of 300 mg of Irbesartan to usual antihypertensive therapy induced a mean (95% CI) reduction in albuminuria of 37 (20 to 49) %, $p < 0.001$ (geometric mean (95% CI) from 1574 (1162 to 2132) to 996 (699 to 1419) mg/24 h), a reduction in 24-hour blood pressure of 8 (-2 to 18) / 5 (1 to 9) mm Hg, $p = 0.11$ / $p = 0.01$ (mean (SE) from placebo 146(4) / 80 (2) mm Hg). GFR remained unchanged. Linear regression revealed: Reduction in albuminuria (%) = $2.3 \times$ reduction in diastolic blood pressure (mmHg) + 18, $p = 0.015$, $r^2 = 0.32$. S-potassium increased ((mean (SE)) 4.3 to 4.6 mmol/L, $p = 0.02$). Intervention to reduce s-potassium was needed in 2 patients, both with GFR below 35 ml/min*1.73m². Otherwise the combination therapy was safe.

Conclusions: Dual blockade of the RAS offers additional renal and cardiovascular protection in type 1 diabetic patients with DN resistant to conventional antihypertensive treatment including maximally recommended doses of ACEi.

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Diabetic Foot: Pathogenesis and Charcot

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BONE MINERAL DENSITY IS REDUCED IN DIABETIC PATIENTS WITH CHARCOT ARTHROPATHY.

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Background and Aims: To determine if bone mineral density (BMD) is reduced in patients (pts) with diabetes mellitus (DM) with Charcot arthropathy and to compare/contrast the BMD in pts with Type 1 (T1) and Type 2 (T2) DM. **Materials and Methods:** Retrospective chart review of 24 DM pts with Charcot arthropathy. All BMDs were performed on a Hologic 4500 A.

Results: 11 T1DM pts (6F) and 13 T2DM pts (8F) had median age (47 yrs/58 yrs); DM duration (24 yrs/18 yrs); and BMI (25.9/31.8). Advanced complications were noted in T1 and T2 DM pts as follows: peripheral neuropathy (100%/100%); retinopathy (100%/39%); proteinuria (82%/62%); hypertension (64%/69%); dyslipidemia (55%/62%); and macrovascular disease (27%/46%). Cardiac autonomic neuropathy was noted in 73% of T1DM pts and 31% of T2DM pts. BMD results appear in the table below with median scores reported.

	A-P Spine BMD		Femoral Neck BMD		Total Hip BMD	
	g/cm ³	T-Score	g/cm ³	T-Score	g/cm ³	T-Score
T1DM	0.910	-1.27	0.649	-2.51	0.764	-1.94
T2DM	1.242	1.51	0.754	-1.53	0.994	-0.25

T-scores: Osteoporosis ≤ -2.50 ; Osteopenia $-2.49 \leq x \leq -1.00$; Normal ≥ -0.99

Femoral neck BMD was reduced in both groups, with the median T-score in the T1DM pts in the osteoporotic range and that in the T2DM pts in the osteopenic range. At all 3 anatomic sites, BMD was lower in T1DM pts.

Conclusion: Low bone mass was noted in T1DM pts with Charcot arthropathy at all 3 sites, but only at the femoral neck in T2DM pts with Charcot arthropathy. DM pts with Charcot arthropathy tend to have long-standing DM and advanced DM complications. In this population, it remains to be determined if early diagnosis of low BMD and initiation of antiresorptive therapy will be beneficial in the prevention of either recurrent Charcot or common osteoporotic fractures.

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EARLY DIAGNOSIS OF CHARCOT OSTEOARTHROPATHY: THE OPPORTUNITY TO ARREST ITS DEVELOPMENT

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Background and Aims: Early diagnosis of Charcot osteoarthropathy to prevent deformity is important. A high index of suspicion is needed. The aim of this study was to diagnose Charcot osteoarthropathy as early as possible and initiate early treatment.

Materials and Methods: We studied 21 diabetic patients presenting with a red, hot, swollen foot, mean age 52 ± 10 years (mean \pm SD). Initially, each patient underwent X-ray and technetium hydroxy-diphosphonate scan with X-ray follow-up, in order to evaluate possible further changes. All patients were treated with offloading including combinations of total contact casts (6), Aircasts (13) and crutches (15) and wheelchairs (10).

Results: In 10 patients, the X-ray revealed classical changes of Charcot's osteoarthropathy, 5 in the mid-foot and 5 in mid-foot and hindfoot. The bone scan showed increased uptake in these areas. In 5 patients, the radiological changes were borderline, 2 in the mid-foot, 2 in the mid- and hindfoot and one in the hindfoot. However the bone scan showed markedly increased uptake of isotope not only in the area of the borderline radiological abnormality but throughout the mid-foot or the hindfoot or both, confirming the diagnosis. Radiological follow-up at 3 months revealed Charcot changes in a similar distribution to that of the bone scan. In 6 patients, the X-ray was normal at the time of presentation. However the bone scan showed focal hot spots, 4 in the mid-foot and 2 in the hindfoot. In 2 cases, radiological changes of Charcot developed at 2 and 3 months respectively. However, in 4 patients, the X-ray remained unchanged, at a mean follow-up of 2.1 ± 1.4 years.

Conclusions: It is now possible to diagnose the Charcot foot at a very early stage with abnormal bone scan but normal X-ray to prevent progression of this condition.

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THE BIOMECHANICAL FEATURES OF CHRONIC STAGE OF CHARCOT FOOT AND THEIR CHANGES AFTER ONE YEAR OF FOLLOW-UP

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Background and aims: Charcot arthropathy (CA) is believed to be one of the serious complications of diabetic neuropathy. Progressive deformation of the foot in patients with CA creates biomechanical abnormalities that lead to ulceration and amputation. The aim of present study was to investigate the biomechanical parameters of the feet with chronic stage of midfoot CA and their changes after one year of follow-up. **Patients and methods:** Fifteen diabetic patients (5 males, 10 females) with CA in midfoot («rocker bottom» or «medial convexity») were examined. Mean age was 51.7 ± 13.2 years, duration of diabetes - 23.4 ± 9.6 years. Five patients were re-examined after one year. The absence of active stage was confirmed by X-ray, 10-g monofilament and biothesiometry in conjunction with plantar pressure distribution measurement (emed-AT-2, novel GmbH, Munich) were carried out. Data analysis was performed with novel scientific software. Peak pressures (PP), N/cm², maximum force (MF), % of body weight (BW), force-time integrals (FTI), % of BW \times s for different foot areas, arch indexes (AI), and other geometric parameters were calculated. **Results:** AI (0.32 ± 0.05 vs 0.21 ± 0.09), PP (39.0 ± 32.5 vs 10.3 ± 5.6), MF (46.2 ± 25.0 vs 16.3 ± 11.4) and FTI (30.5 ± 18.9 vs 9.1 ± 7.9) under the midfoot with CA were increased ($p < 0.05$) compared to the contralateral foot. Subarch angle in CA feet was also greater (114.1 ± 2.8 vs 94.4 ± 7.0). After one year of follow-up the significant changes in PP were found only in two patients with PP under the midfoot greater than 60 N/cm^2 : 94.6 ± 9.9 vs 59.8 ± 7.6 and 99.2 ± 16.6 vs 72.7 ± 4.9 . Highest PP remained unchanged in one patient: 110.9 ± 14.4 vs 103.4 ± 12.6 and no changes were observed in two patients with PP less than 60 N/cm^2 . **Conclusions:** Charcot arthropathy in midfoot is characterized with high level of midfoot loading and increased subarch angle. Changes in the PP during follow-up in chronic CA appear to depend on the baseline PP level.

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Autonomic, peripheral neuropathy and osteoporosis in the diabetic foot

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Aims: To correlate the bone mass density (BMD) in the calcaneum in patients with and without neuropathic diabetic foot (NDF) and patients with autonomic neuropathy (AN). **Material and methods:** 60 type 1 DM patients were evaluated and divided into three homogeneous groups: I – without NDF (group 1), II – with NDF (group 2), III – with NDF and AN (group 3). Group 1: 18 patients (8 male and 10 female) mean age 40.7 ± 8.9 yrs. Group 2: 20 patients (m/f: 10/10) mean age 46.9 ± 11.5 yrs. Group 3: 22 patients (m/f: 10/12) mean age 53.6 ± 9.1 yrs. The average of HbA1c, BMI and duration of diabetes were also evaluated. BMD was measured at the distal radius by Dual Energy X-ray Absorptiometry (DEXA) and on the left calcaneum by Ultrasonography. **Results:** in group 1 the averages were: HbA1c (9.0 ± 1.8); BMI (24.9 ± 1.7) and duration of DM (8.6 ± 5.8). BMD at the distal radius was normal in 16 patients (88.9%) osteopenia in 2 patients (11.1%). BMD on the calcaneum was lower in 2 patients (11.9%) while 16 patients showed normal values (88.9%). In group 2: the averages were: HbA1c (9.3 ± 2.1); BMI (25.9 ± 1.5) and duration of DM (14.6 ± 7.6). BMD at the distal radius was normal in 8 patients (40%) osteopenia in 9 patients (45%) and osteoporosis in 3 (15%). BMD on the calcaneum was lower in 4 patients (20%) while 16 patients showed normal values (80%). In group 3: the averages were: HbA1c (9.7 ± 1.9); BMI (26.4 ± 1.7) and duration of DM (16.6 ± 7.2). BMD at the distal radius was normal in 6 patients (27.3%) osteopenia in 11 patients (50%) and osteoporosis in 5 (22.3%). BMD on the calcaneum was lower in 7 (31.8%) while in 15 patients showed normal values (68.2%). The difference in BMD on calcaneum was statistically significant between 1 and 2 (11.9% VS 20% $p = 0.01$); between 2 and 3 (20% VS 31.8% $p = 0.01$) being the higher degree of osteoporosis in the group with NDF and AN. **Conclusions:** 1. Peripheral neuropathy could be a risk factor for osteoporosis in the diabetic foot. 2. Association autonomic neuropathy significantly increases the risk of osteopenia development in the diabetic foot.

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THE EFFECT OF POLYNEUROPATHY ON THE FOOT MICROCIRCULATION IN TYPE 2 DIABETES

Nabuurs M, Houben A, Dennert J, Bleeker D, de Bruin T, Schaper N. Dept. of Int. Medicine, university hospital Maastricht; CARIM, Maastricht, The Netherlands. **Background and Aims:** Peripheral polyneuropathy (PNP) is a risk factor for diabetic foot ulcers. Polyneuropathy could result in impaired peripheral microcirculation, jeopardising delivery of fluid and nutrients. Therefore, we studied microcirculation and fluid filtration rate (FFR) in the feet of type 2 diabetic patients with PNP (PNP+, n=14) and with a history of a neuropathic foot ulcer (Ulcer, n=9), without PNP (PNP-, n=14), and 14 healthy controls (C). All subjects were matched for age, gender, BMI, and were without peripheral vascular disease or varicosis. **Materials and Methods:** FFR was measured by mercury strain gauge plethysmography, skin blood flow by laser-Doppler fluxmetry (LDF) and capillary blood cell velocity (CBV)/capillary density by nailfold capillary microscopy. All measurements were performed first supine and subsequently during 50 minutes sitting in standardised conditions. **Results:** Supine CBV (data not shown) and Effective Nutritive Blood Flow (ENBF = CBV x capillary density) were lower in Ulcer (6.6 number/mm/s) compared to PNP+ (13.9), PNP- (16.4), and C (12.1) ($p=0.05$). In the diabetic patients we found a negative correlation between the severity of neuropathy (Valk-score) and ENBF (supine $r=-0.46$; $p<0.02$). After sitting the decrease in LDF was larger in Ulcer (-33%) compared to PNP+ (-10%), PNP- (-14%), and C (-1%) ($p=0.005$). No further differences in microcirculatory variables were observed. Compared to Controls (0.00286 ml/dl/s) FFR in the first 10 minutes sitting was lower in Ulcer (0.00121) and PNP+ (0.0020) ($p<0.001$) and unaltered in PNP- (0.00194). Furthermore, we found a negative correlation ($r=-0.41$; $p<0.01$) between Valk-score and FFR in the first 10 minutes of sitting. **Conclusion:** In type 2 diabetic patients skin microcirculation and fluid filtration are in particular impaired in subjects with a history of foot ulceration. These defects are probably related to polyneuropathy, as the decrease in ENBF and FFR were associated with the severity of PNP. These results suggest that PNP results in a decrease in total microcirculatory blood flow with a subsequent loss of capillary filtration surface. Alternatively, the decrease in FFR could be caused by an increase in interstitial hydrolic pressure in the neuropathic foot, as observed earlier.

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PREVALENCE OF PATHOGENIC ORGANISMS IN SEVERE FOOT INFECTIONS IN THE OUTPATIENT DIABETIC FOOT CLINIC.

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Background and Aims: Foot infections are a common and serious problem in diabetic patients. The aim of this work was to study the prevalence of pathogenic organisms in severe foot infections and to assess the efficacy of the empirical antibiotic regimens.

Materials and Methods: A retrospective analysis (between Nov/97 and Dec/99) of 78 infected foot ulcers was done. 91% of the patients had type 2 Diabetes and 9% type 1 Diabetes. The mean age was 62 years (min-max; 28-85) with 49% females and 61% males. The mean time of evolution since diagnosis was 15 years (1-41). 73,1% had a neuropathic foot and 26,9% had a vascular foot. We have found radiological and/or probe to bone evidence of osteomyelitis in 53,8%. The samples were obtained from the deeper tissue, and by needle aspiration when pus was present.

Results: 78 cultured wounds were positive and studied. 23% of the cultured wounds were polymicrobial, with 44,4% of anaerobes. Gram(+) aerobic bacteria were the commonest micro-organism isolated (51,3%), followed by Gram(-) aerobic bacteria (25,7%). Anaerobes were isolated in 30,8%. Of the Gram(+) aerobes, *Staphylococcus aureus* was found most frequently (67,5%) and 26% were methicillin-resistant (MRSA). MRSA was isolated only in patients treated with several antibiotics before. *Pseudomonas aeruginosa* was present in 15,3% of the Gram(-) aerobes isolations. All patients initiated empirical treatment. The antibiotics selected were amoxicillin/clavulanate (29,5%), ciprofloxacin/clindamycin (29,5%) and imipenem (41%). Of the micro-organisms isolated, 17 were resistant to the antibiotic selected. These included: 7 MRSA, 5 *Pseudomonas aeruginosa*, 4 *Morganella morganii* and 1 *Serratia marcescens*. These micro-organisms were more resistant to imipenem ($p=0,0011$) and less to ciprofloxacin/clindamycin ($p=0,052$). One of the outcomes of the empirical treatment was a major amputation in 8 patients. These amputations were more frequent when resistant micro-organisms were isolated ($p=0,058$).

Conclusions: The prevalence of the micro-organisms isolated is similar to that described in the international studies, with a significant number of MRSA, but with less polymicrobial isolations. The revision of the initial antibiotic regimens according to antibiograms is mandatory to ensure better outcomes.

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PERIPHERAL AUTONOMIC NERVOUS SYSTEM FUNCTION AND FOOT ULCERATION IN DIABETES

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Background-Aims: Although peripheral sensory neuropathy (PN) is a major risk factor for foot ulceration, there is paucity of data concerning the contribution of impaired peripheral autonomic nervous system (ANS) function to foot ulceration in diabetic subjects (DS). This study examined the relationship between indices of ANS function and foot ulceration in diabetes. **Materials and Methods:** Forty-nine patients with either type 1 or type 2 diabetes were studied. Diagnosis of PN was based on clinical symptoms (NSS), signs (NDS) and quantitative sensory testing (vibration perception threshold, VPT). DS were matched for age and sex and divided in 3 groups: Without PN (DN-, n=22); with PN (DN+, n=13); with neuropathic foot ulceration (DFU, n=14). Postural vasoconstriction arteriolar reflex (VAR) and ANS activation during deep breathing (DB) were evaluated using laser-Doppler flowmetry at the dorsal aspect of the right foot. Sympathetic skin response test (SSR) was also performed on the same foot. **Results:** VAR [median (interquartile range)] (%) values were not significantly different among DN-, DN+ and DFU [36.6 (28.7-53.3), 23.8 (1.2-42.3) and 43.7 (15.5-61.6) respectively, $P=0.19$]. The same was valid for DB values (%) [38.1 (22.7-48.6), 20.3 (13.9-33.8) and 29.9 (10.9-40.6) respectively, $P=0.08$]. SSR was absent in all but 2 DS with DFU, in 6 DS in the DN+ group and in 2 DS in the DN- group ($P<0.0001$). Logistic regression analysis, adjusted for age, sex, blood pressure, duration and control of diabetes, as well as the indices of peripheral somatic and ANS function, showed that only HbA_{1c}, NDS, VPT and an absent SSR, but not VAR or DB, are associated independently with foot ulceration: Odds ratio (95% confidence intervals): 1.48 (1.04-2.10), 2.67 (1.50-4.75), 1.20 (1.08-1.33), 3.75 (1.24-11.24), 1.01 (0.99-1.03) and 0.98 (0.94-1.02) respectively. **Conclusions:** Poor diabetes control, severity of peripheral somatic neuropathy and absence of sweating-but not impaired vasoconstrictor tone-in the feet are associated with increased risk for foot ulceration in diabetic patients.

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Assessment of non-specific immune response to infection in patients with diabetic foot

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Background and Aims: The major factors of a non-specific immune response are phagocytic cells including polymorphonuclear leukocytes (PMN), which are also able to kill and degrade the substances which they eat in a process requiring energy and associated with a 'respiratory burst' of oxygen consumption. The aim of our study was to assess PMN functions in patients with chronic diabetic foot (DF) bacterial infection.

Materials and Methods: 30 patients treated over one month for an infected DF in our foot clinic (mean age 54 ± 8 years, duration of diabetes 20 ± 9 years, HbA_{1c} 8.8 ± 1.5 %) were matched with 25 healthy controls. All patients were without clinical signs of acute deep infection and without critical leg ischemia. Phagocytosis and respiratory burst of PMN were determined by flow cytometry (Phagotest, Bursttest; Orpigen Pharma, Heidelberg, F.R.G.) in basal state and after stimulation with *Escherichia coli*.

Results: The patients with DF did not differ significantly in the count of active PMN in basal state in Bursttest from healthy controls (2.6 ± 2.0 vs. 3.0 ± 2.8 %), but the fluorescent activity of these cells was significantly lower in the group of patients with DF (396 ± 228 vs. 574 ± 337 , $p < 0.05$). There were no significant differences in the count of active PMN (87 ± 13 vs. 82 ± 19 %) and their fluorescent activity (3097 ± 2122 vs. 3270 ± 2135) in stimulated state between the group of patients with DF and healthy controls in Bursttest. The count of phagocytizing PMN in basal and stimulated state and their fluorescent activity did not differ significantly between both study groups.

Conclusions: The results of our study show a slightly altered non-specific immune response to infection in diabetic patients with chronic foot infection in comparison with healthy subjects.

Supported by grant of Ministry of Health IGA No. 5223-3

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Measurement of toe blood pressure is useful in screening for lower extremity arterial disease in diabetes patients.

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Background and Aims: Screening for lower extremity arterial disease (LEAD) has often included measurement of ankle blood pressure (AP) and ankle/arm index (AI). However, in diabetes patients the readings are often higher than the true pressures due to medial sclerosis. Therefore, we evaluated a screening program using toe blood pressures (TP) and toe/arm index (TI) measured with a simple non-invasive method.

Materials and Methods: We recruited 437 subjects (age 30-70) none of whom had any previously known or suspected LEAD. 134 subjects were controls (C), 166 had type 1 (T1D) and 137 type 2 (T2D) diabetes. A blood pressure cuff on the ankle or the proximal part of the great toe was connected to a sphygmomanometer and the distal pulse was monitored with Doppler for AP and a pulse oxymeter sensor for TP. Impaired peripheral circulation was defined as pressures or indices below mean-2SD for the control group.

Results: When using AP, AI (a tib ant and a tib post), TP and TI together, 6% of C, 24% of T1D and 31% of T2D had at least one of those measurements below normal in either leg, i.e. a sign of impaired arterial circulation ($p<0.001$ for T1D or T2D vs C). With AP and AI alone these numbers were 4%, 10% and 12%, respectively ($p<0.05$). Low TP (<79 mmHg) or TI (<0.74) despite normal AP and AI was found in 2% of C, 16% of T1D and 20% of T2D ($p<0.001$). Conversely, low AP or AI combined with normal TP and TI was found in only 4%, all groups included. Furthermore, the number of diabetic subjects exhibiting low AI or TI was 4-5-fold greater than those with low AP or TP.

Conclusions: In diabetic patients, measurement of TP and TI, as compared to AP and AI alone, markedly improves the diagnostic sensitivity with respect to LEAD, and this is probably mainly explained by medial sclerosis being common at the ankle but not at the forefoot level. Furthermore, reduced AI and TI, i.e. indices adjusting for systemic blood pressure were more sensitive than the absolute pressures (AP and TP) alone. We suggest that toe blood pressures should be included in the evaluation of atherosclerotic disease in diabetes. 25-30% of asymptomatic patients may exhibit impaired peripheral circulation.

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Increase in body weight contributes to increased mean peak plantar foot pressures and pressure times in patients with diabetic neuropathy

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Background and Aims: Foot ulcers have been shown to occur at sites of high plantar pressure in patients with diabetic neuropathy. The most common anatomical sites of foot ulcerations are under the metatarsal heads and the plantar aspect of the big toe. The aim of this study was to investigate the peak plantar pressures and pressure times on the above anatomical sites under the effect of increased weight in patients with diabetic neuropathy.

Materials and Methods: Two groups of volunteer's patients with type II diabetes were evaluated. Group A ($n=10$) composed of patients with diabetic neuropathy ($VPT>25$ volt, 5.07 S.W. monofilament 10-g insensitivity). Group B ($n=10$) composed of patients without neuropathy. The subject groups were of similar age, sex, BMI and duration of diabetes. Using the Foot-Scan RS International barefoot pressure measurement system, repeated measures were recorded on both groups and peak plantar pressures and pressure times were compared under three conditions. The first condition involved measurements without any additional weight and served as a baseline measurement (C1). The second and third test conditions involved pressure measurements with an additional 5 kg (C2) and 8.5 kg (C3) respectively, of weight evenly distributed in pockets of a workout vest. For the purpose of the study, only data recorded from under the metatarsal heads and big toe were used for analysis, and the mean peak pressures in N/cm² (MPP) and mean pressure times in msec. (MPT) were obtained. All patients were able to walk comfortably, unaided, at their own pace.

Results: In group A there was a significant increase in mean peak plantar foot pressures for each incremental increase of weight (MPP: C1=16.09; C2=17.21; C3=18.81 that C1 vs C2: $p<0.05$; C1 vs C3: $p<0.05$ and C2 vs C3: $p<0.05$). The mean peak pressure times were significant increase in C3 (MPT: C1=563.2; C2=579.9; C3=635.31 that C1 vs C3: $p<0.05$ and C2 vs C3: $p<0.05$). In group B there was not any significant statistical differences between the three conditions (MPP: C1=17.39; C2=17.50; C3=17.33 and MPT: C1=609.6; C2=620.7; C3=624.5).

Conclusions: Small amount of increase in body weight contributes to increased mean peak plantar pressures, while bigger amounts of increase in body weight contributes also to increase mean peak pressure times in patients with diabetic neuropathy.

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Diabetic Foot: Epidemiology

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Regional differences concerning diabetic foot lesion outcomes - preliminary results of a prospective study

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Background: Although the pathogenesis of the diabetic foot is probably similar in different parts of the world, regional variation concerning proportions of underlying risk conditions and clinical presentation has been described. Differences concerning outcome of those lesions are to be expected.

Aim: To determine the differences of diabetic foot lesion outcomes in different regions of the world. Patients and Methods: 613 consecutive patients with diabetic foot lesions from three centers (A=Soest/Germany, B=Dar es Salaam/Tanzania, C=Chennai/India) were included in this study. Outcome related data (healing without amputation, performed amputation, or death before healing), as well as data on event duration were collected for each patient. In addition, the impact of peripheral vascular disease (PVD) on outcome was investigated.

Results: Complete information was available on 451 patients (74%). PVD was evident among 50% of the patients from A, 22% from B, and 17% from C. Revascularization was implemented in 14% of the German patients, but not in centers B and C. In all three centers, healing without amputation was attainable in more than two thirds of the lesions (76%, 73% and 77%). Amputation had to be performed in 20.2% (Major: 7.1%), 22.5% (7.9%) and 22.7% (4.5%) of the cases, respectively. Average healing duration was 92.2 days in A, 132.3 days in B, and 118.3 days in C. While only 7.9% of patients without evidence of PVD from center A underwent amputation, it was far more frequent in B in C (18.8% and 18.7%, $p<0.05$). Tanzanian patients with PVD had an almost ninefold increased risk of death prior to healing compared with their counterparts without vascular disease (35% and 4%, respectively, $p<0.001$).

Preliminary Conclusion: In the German population, the outcome of diabetic foot lesions seems to be predominately influenced by the presence and successful therapy of PVD. In contrast, progressive infection appears to be the primary problem among developing countries, as reflected by high amputation rates in patients without evidence of vascular disease.

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Is it better if we show a fall, or a rise, in the rate of major amputation?

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In the very long term, it is to be expected that improvement in diabetes management will lead to a substantial fall in the number of major (at or about the knee) amputations undertaken for diabetes. This does not mean, however, that amputation rate is necessarily a guide to the quality of specialist foot care in the short term. This is because the principal reasons for variation in amputation rate relate not to the quality of management but to the nature of the population, the structure of health care services, and prevailing medical orthodoxy. Amputation rate is a reflection of medical activity, and not just of disease incidence and severity. If a sizeable percentage of foot ulcers are also managed by non-specialist teams, it follows that the rate of amputation may be largely beyond the influence of specialists working within the field. Our own unpublished observations made at the time of the release of the St Vincent Declaration targets in 1989, indicated that only about 25% of the expected major amputations for diabetes were initiated by our multidisciplinary foot care clinic. We believed therefore that the primary target for the care of established foot problems should be to ensure a greater referral rate to specialist services, and that within those services this should be reflected in a rise in amputation rate, and not a fall.

We have therefore analysed data collected in our unit on both minor and major amputations between 1988 and 1999. The mean annual rate of referral of patients with new ulcers has increased progressively from 40.0 (1988-1990) to 87.3 (1997-1999). Over the same ten year interval there was a rise in the mean annual number of major and minor amputations from 5.0 and 5.7, respectively, to 9.3 and 16.3. However, when expressed as a percentage of new referrals, there was no change in either: 12.5 and 14.3% to 10.6 and 18.6%. These data confirm that we have substantially improved the percentage of foot ulcers being managed by specialist services, and this is reflected in a substantial rise in the number of amputations performed.

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Non-traumatic lower extremity amputations in diabetic patients: a 8-year population based survey in an Italian region.
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Background and Aims: To perform a retrospective, population-based survey of all non-traumatic lower-extremity amputations (LEAs) carried out in Umbria (831,714 inhabitants), Italy, in 8 years (1991-1998).

Materials and Methods: The registries of the operating rooms of all the surgical wards in Umbria were examined to quantify the LEAs and identify the subjects. Details about the patients were subsequently obtained from the hospital records of all the amputees (A). At least one of the following criteria had to be fulfilled to identify a patient as diabetic: 1) history of diabetes, 2) pharmacologic hypoglycemic therapy before surgery, and 3) fasting plasma glucose >140 mg/dl before surgery.

Results: In 1991-1998, 973 umbrian residents underwent LEAs and 52.2% of them were diabetics. The median age at LEA was 74 yrs (range 35-96) for diabetic A (DA) and 78 yrs (44-98) for non diabetic A (NDA) ($p<0.001$). Within both groups age at LEA was higher in females than in males. Males were more numerous than females among the A, but the sex ratio (M/F) within the groups was markedly different (1.3 in DA and 2.3 in NDA; $p<0.001$). The incidence of new A was 22.84/10,000/yr in diabetics and 1.06/10,000/yr in non diabetics. The incidence increased with age in both groups and was significantly higher in diabetics at all ages. Overall perioperative mortality was 8.8% and was higher among the NDA (10.5% vs 7.3%; $p=ns$). The number by year on new DA did not significantly change over the 8-year period. However, the ratio between major (above ankle) and minor (below ankle) new DA progressively decreased from 1.41 to 0.61 suggesting that LEAs are now performed at a more distal level than in the previous years.

Conclusions: although in our region DA still represent more than 50% of all the A and their number did not decrease over the years, the level of amputation is now more distal than in the past.

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DIABETIC FOOT SYNDROME (DFS) RISK FACTORS AND INCIDENCE IN THE DIABETICS, RESIDENTS OF UZBEKISTAN.
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AIMS: to assess diabetic foot syndrome risk factors and incidence in patients with diabetes mellitus (DM), residents of Uzbekistan. **MATERIALS AND METHODS:** 1063 DM patients (555 females, 508 males, 36.5% with 1 type DM, 63.5% with 2 type DM) referring to the Institute of Endocrinology within a year were examined by a podiatrist for assessment of vibration sensation by a calibrated tuning fork, of tactile perception by a 10g. monofilament, of thermal sense by TRI-TERM device as well as of pain sensitivity and tendon reflex. Glycemia and HbA1c level were measured to rate compensation, Wagner classification being used to value diabetic ulceration. **RESULTS:** peripheral polyneuropathy was diagnosed in 74% of DM patients (23.6% with 1 type DM, 76.4% with 2 type DM). DFS was observed in 25% of the diabetics (33.1% and 66.9%, respectively), DFS neuropathic form making it 82.7%, the ischaemic one – 1.6%, the mixed one – 15.7%. Risk factor analysis showed DFS incidence of 12.5% and 69.8% depending on the disease duration of < 5 years and > 10 years, DFS incidence of 6.5% and 42.7% in age groups of 20-29 and 60-69 years, respectively. HbA1c level in DFS patients was more than 11%, that is, confidently higher ($p<0.001$) than in the group without DFS. **CONCLUSIONS:** DFS was observed in 25% of DM patients admitted at the Institute of Endocrinology, disease duration, age and diabetes compensation stage being the major risk factors. High DFS incidence, resulting in disability of DM patients, makes necessary self-control optimization as well as diabetic foot departments in various areas of Uzbekistan and training of podiatrists for diabetic foot intervention measures to be accomplished.

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Prevalence and Risk factors for Peripheral Vascular Diseases among Korean diabetic patients

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Background and Aims: It has been reported that the prevalence rate of peripheral vascular diseases of the Western diabetics is 20-40% while that of the Asian counterparts is low at about 3-6%. Hence, this study investigated the prevalence and the risk factors of peripheral vascular diseases among Korean diabetics.

Materials and Methods: This study included 1,677 and 850 patients at each of the respective two hospitals-one in Seoul and one Suwon (a city located in the vicinity of Seoul). Endocrinology outpatient clinics were visited for three months from December 2000 through February 2001. Among them, 1316 patients (52% of the participated patients) had their ankle-brachial index measured. Peripheral vascular disease was defined as having an ankle-brachial index of < 0.9. And it investigated conventional risk factors of atherosclerosis and presence of diabetic complications.

Results: The prevalence rate of peripheral vascular diseases was 2.5% (33 cases). The prevalence rate of patients with ABI<0.95 was 4.9% (65 cases) while that of the patients with ABI<1.0 was 6.8% (90 cases). When these two groups (ABI<0.9 vs ABI>=0.9) were compared for the presence of a peripheral vascular disease, a significant difference was merely observed with respect to age, systolic and diastolic blood pressure (67.8+/-10.9 vs. 59.2+/-11.5 years old, $p=0.0001$; 145.3+/-16.1 vs. 130.9+/-17.7 mmHg, $p=0.0001$; 83.0+/-9.1 vs. 79.0+/-10.5 mmHg, $p=0.018$, respectively). The results of the stepwise logistic analysis showed that age (OR, 0.934, $p=0.014$) and systolic blood pressure (OR, 0.965, $p=0.008$) were significant risk factors.

Conclusions: Unlike the Western counterparts, Korean diabetic patients showed a low prevalence rate of peripheral vascular disease, revealing a similar prevalence rates as that of other Asian populations. Among Korean diabetics, the age and systolic blood pressure were the most significant risk factors of peripheral vascular disease.

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INCIDENCE OF TYPE 2 DIABETIC FOOT ULCERATION AND LOWER LIMB AMPUTATION IN A DUTCH PRIMARY HEALTH CARE SETTING
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Background and Aims: Studies outside the Netherlands show varying prevalences of diabetic foot ulceration from 2-7% and of lower limb amputation from 1.3-4%. Nothing is known about its incidence. Our aims are to determine the incidence of diabetic foot ulcers and lower limb amputations in type 2 diabetic patients in a primary health care setting. **Materials and Methods:** The data were collected in the academic research network of the department of General Practice University Nijmegen (the Nijmegen Monitoring Project) between 1993 and 1998. The NMP is a continuous prospective registration of the course of type 2 diabetes mellitus in 9 general practices, with a total population of 46,500 patients. In addition chart research was performed in all patients with registered diabetic foot problems, those who died, moved to a nursing home or were under specialist care. The yearly incidence of foot ulceration was defined as the number of type 2 diabetic patients who developed a new foot ulcer. Likewise the incidence of lower limb amputation was defined. **Results:** The study population of type 2 diabetic patients per year increased from 552 patients in 1993 to 745 patients in 1998. The yearly incidence of foot ulceration varied from 1.1-2.7% with a mean of 1.9%. 25% of the patients had recurrent episodes. The yearly incidence of patients with an amputation varied from 0.4-0.8% with a mean of 0.5%. Ten of the 15 patients with a lower limb amputation died, 4 patients during their hospital stay for amputation, the other 6 between one and 3 years after their first amputation. In 80% foot ulceration preceded the amputation. **Conclusions:** The incidence of diabetes related foot ulceration and lower limb amputation is low but persistent in this unselected population. The recurrence rate and risk for subsequent amputation are high with devastating consequences of invalidation and a high mortality.

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REPRODUCIBILITY OF FOOT EXAMINATION IN DIABETIC FOOT PATIENTS

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Background and Aims: Foot examination is the cornerstone for foot ulcer prevention and several techniques have been advocated to detect patients at risk. However, how these techniques perform in elderly diabetic patients in daily practise frequently remains to be determined. In the present study we investigated the intra- and intertester reproducibility (*Intra* and *Inter*) of commonly used techniques. **Materials and Methods:** To determine *Intra* 45 diabetic patients, mean age 67.4 yrs., with polyneuropathy, were examined twice by the same examiner. To determine *Inter*, 20 identical patients, were examined by 2 examiners. All examinations were performed on 2 separate occasions and consisted of: pinprick (Neurotip); monofilament (10 gram on apex hallux); tuning fork (128 Hz on apex hallux); Neuropathy Symptom Score (NSS-questionnaire); toe pressure (ToeP: pletysmography); Ankle-Brachial-Index (ABI: Doppler); foot type (FT: normal, flat or hollow); metatarsal length (ML: relative length of metatarsals in relation to each other); arterial pulsations foot; length and width of shoes; weight-bearing goniometry of the MTP1 (G-MTP1) and ankle joint (G-angle). Reproducibility of each measurement was scored as: <.40 = poor; .40 - .60 = questionable; .60 - .80 = reasonable; > .80 = good. **Results:** *Intra* was questionable in ML (.44) and FT (.56); reasonable scored ABI (.64), art. pulsations (.68), ToeP (.74), G-angle (.74), pinprick (.75). Good scored: shoe examination (.83), monofilament (.87), G-MTP1 (.80), NSS-score (.89) and tuning fork (.92). *Inter* was poor in ML (.33) and FT (.34); questionable were ABI (.56) and art. pulsations (.56). Reasonable scored: ToeP (.66), G-angle (.67), pinprick (.69), NSS (.76), monofilament (.77), shoes examination (.77) and G-MTP1 (.78). Good scored tuning fork (.83). **Conclusions:** Intertester reproducibility was higher than intratester in most examinations. Determination of foot type, palpation of metatarsal heads, palpation of arteries and ankle pressure measurement probably have a too low reproducibility for use in daily clinical practise. Acceptable reproducibility had the tests for neuropathy, toe-pressure measurement and shoe examination. In contrast to earlier studies, in which joint mobility was tested passively, the reproducibility of joint mobility tested during weight bearing was acceptable for daily practise.

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EPIDEMIOLOGICAL STUDY OF THE INDICATORS RELATED TO DIABETES FOOT PROBLEMS IN BLACK SEA AREA

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Background and Aims: To demonstrate effective routine implementation of modern information and communication technology in the Black Sea area for the epidemiological study of the foot complications of diabetes mellitus, the most prevalent and costly of all the diabetes-related complications. **Materials and Methods:** To obtain a usable data set for audit, patient data were standardised on the WHO/EUROPE recommended Basic Information Sheet diabetes dataset. According to St. Vincent Declaration the most important factors related to the development of foot ulcers are: regular inspection of foot, to identify whether or not is peripheral neuropathy, normal vibratory sensitivity, normal pin prick sensitivity, foot pulse present, healed ulcers, acute ulcers/gangrene, bypass/angioplasty leg amputations above ankle and leg amputations below ankle. The objectives of the Black Sea Tele Diab System (BSTD) were to develop and evaluate the use of a fully-computerised healthcare record system in a clinical setting, to promote the use of electronic data exchange of healthcare information and to provide a new framework for the epidemiological study and monitoring of diabetes care. Data of 3236 diabetics (mean duration 9.54 ± 8.35 yrs, 1161 diabetics Type 1 and 2075 diabetics Type 2) were collected in diabetes clinics using the BSTD system from diabetes centres in Romania, Ukraine and Rep. of Moldova. All analysis were done with SPSS 10 for windows 2000. **Results:** 2739 patients (84.6 %) had regular inspection of the foot during the last 12 months, among them 1381 patients (42.7 %) had peripheral neuropathy, 919 patients (28.4 %) had no normal pin prick sensitivity, 927 patients (28.6 %) had no normal vibratory sensitivity, 335 patients (10.4 %) had no foot pulse present, 139 patients (4.3 %) had healed ulcers, 49 patients (1.5 %) had acute ulcers/ gangrene, 15 patients (0.5 %) had bypass/angioplasty, 23 patients (0.7 %) had above ankle leg amputations, 22 patients (0.7 %) had below ankle leg amputations. **Conclusions:** (1) The BSTD software project has succeeded in delivering, free available, a competent piece of diabetes clinic software, in step with emerging European standards and the St. Vincent declaration and to ensure that all patients are adequately monitored. (2) There are some BS regions which are not covered effectively by sufficient number of cases i.e. the case power is not sufficient for diabetes foot problem progression analysis. (3) There is a crucial need for information from these, since it is not possible to make changes to health care delivery or the allocation of resources without data knowledge.

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Factors associated with increased mortality in diabetic patients undergoing amputations

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Background and Aims: Patients with diabetes are at increased risk of amputation and premature mortality. The aim of this study was to identify factors associated with the apparently increased mortality rates among patients with amputations.

Patients and methods: This retrospective study, from 1990 to 2000, compared those patients with a previous amputation (Group A) to an age-matched group without amputation (Group B). Data were collected from a carefully validated population based diabetes information system that employs data linkage to ensure comprehensive capture of amputations and deaths. In group A the metabolic and complications data were from the year of their first amputation. In group B the most recent available data were used.

Results: There were 162 patients in group A, age 71.2 ± 14.6 yrs (mean \pm SD) and 302 in group B aged 70.4 ± 14.4 (p=NS). Seventy-one patients (43.8%) died in group A and 53 patients (17.5%) in group B (p<0.0001). Patients in group A were significantly more likely to be treated with insulin (p<0.0001) and have a longer duration of diagnosed diabetes (18.4 ± 12.2 vs 11.9 ± 7.8 ; p=0.008) compared to group B. Group A had an increased incidence of diabetic complications: neuropathy (54% vs 20%), peripheral vascular disease (70% vs 4%), pre-proliferative and proliferative retinopathy (39% vs 19% and 61% vs 14%, respectively); p<0.0001 for all complications. Furthermore, group A had higher HbA1c (8.9 ± 2.7 vs 8.1 ± 2.3 ; p<0.01), serum creatinine (141 ± 132 vs 95 ± 62 mmol/L; p<0.001), albumin/creatinine ratio (43.5 ± 67.6 vs 6.6 ± 14.9 ; p<0.001) and triglycerides (5.2 ± 19.8 vs 2.3 ± 1.5 mmol/L; p<0.01). Serum cholesterol, systolic and diastolic blood pressure were similar. 35 (21%) patients had more than one amputation of whom 19 (54%) died in the follow-up period compared to 52 (40%) who had a single amputation (p>0.05).

Conclusions: Diabetic patients with amputations do have higher mortality. They also have poorer glycaemic control, higher lipids and worse renal function. Furthermore they have a higher prevalence of all diabetes related complications. Improving metabolic control may help reduce both amputations and mortality in people with diabetes.

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Ethnic Differences in Risk Factors for Diabetic Foot Ulceration.

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Background and Aims: It has been suggested that Asian diabetic patients have a lower prevalence of foot ulceration and amputation than compared to their Europid counterparts. High plantar foot pressure in diabetic neuropathic patients is a major risk factor for foot ulceration and is strongly associated to the amount of subcutaneous plantar tissue thickness. Preliminary evidence has shown lower foot pressures in Asian compared to Europid diabetic patients. Therefore, the aim of this study was to investigate plantar pressures and tissue thickness in these two ethnic groups.

Materials and Methods: Fourteen age and sex matched Asian and Europid diabetic patients with neuropathy but without active or a history of ulceration were studied. Weight bearing plantar tissue thickness of all subjects was measured under each metatarsal head (MTH) during weight bearing and dynamic peak plantar pressure was measured during barefoot walking.

Results: Plantar tissue thickness was significantly greater and peak plantar pressure was significantly lower at all MTHs in the right foot of the Asian patients compared to their Europid counterparts (plantar tissue thickness at MTH 1-5 for Asians vs Europids: 12.2 ± 1.9 mm vs 10.1 ± 1.4 mm, 10.5 ± 2.3 vs 8.2 ± 1.9 , 9.3 ± 1.8 vs 7.4 ± 1.7 , 8.9 ± 1.7 vs 6.9 ± 1.6 and 7.7 ± 2.1 vs 5.4 ± 1.1 respectively, and peak plantar pressure at MTH 1-5 for Asians vs Europids: 381 ± 191 kPa vs 596 ± 245 kPa, 462 ± 291 vs 710 ± 275 , 388 ± 152 vs 580 ± 252 , 278 ± 87 vs 424 ± 281 , 220 ± 170 vs 555 ± 375 respectively). Similar results were obtained for the left foot.

Conclusions: The thickness of the soft tissue under the metatarsal heads was found to be considerably greater in Asian compared to Europid diabetic patients and high plantar tissue thickness was significantly associated with lower foot pressures. This may explain the relative protection of Asian diabetic patients against foot ulceration, a relative uncommon problem in this ethnic group.

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Diabetic Foot: Natural History and Treatment

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Treatment of diabetic foot lesions in hospital: results of 2 consecutive five-year-time periods. A.Benotmane, K. Faraoun, F. Mohammadi, K. Kadi, ME Amani, T. Benkhalifa. Department of Endocrinology and Diabetology, University Hospital of Oran, Algeria

Aims: To compare the rates of limb amputations for diabetic foot lesions, the present study assessed the number of patients with diabetic foot lesions hospitalised in our Department in two five-year-time periods (1989-1993, and 1994-1998), and the percent and site of amputations performed.

Patients and Methods: During the first time period, 132 subjects with 166 lesions (9.16% of the total admissions for diabetes) were hospitalised for diabetic foot lesions, while 176 subjects with 183 lesions (10.57%) were admitted during the second time-period. Patients mean age was similar in 1989-1993 (59.64±11.74 years) and in 1994-1998 (58.25±13.13 years) [NS].

Results: An infectious lesion without neuropathy or arteriopathy was noticed in 6.75% of cases in 1989-1993 and 6.01% in 1994-1998. Most of the foot lesions (63.80% in 1989-1993 and 61.75% in 1994-1998) corresponded either to peripheral vascular disease or both vascular disease and neuropathy. The rest of the lesions (29.45% in 1989-1993 and 32.24% in 1994-1998) corresponded to neuropathic foot ulcers without ischaemia. Limb amputations were undertaken in 30.30% of the patients in 1989-1993, and in 27.84% in 1994-1998 (NS). Minimal amputations with heel preservation were performed in 14.39% of the subjects in the first time-period and in 10.80% of them in the second period (NS). Major amputation rate was similar: 15.91% in the 1st period and 17.04% in the 2nd period (NS). The rate of in-hospital mortality was similar (9.09% and 8.52%, NS) while percentage of patients who left hospital against medical advice increased from 1.52% to 6.82% (p<0.03).

Conclusions: The number of patients with diabetic foot lesions and the rate of limb amputations performed for these lesions did not change significantly over 5 years. The causes of the lack of improvement are multiple: lack of health programmes, absence of structured prevention, and insufficiency of financial resources which is common to developing countries. Civil disturbances can major it, like in Algeria since 1991.

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Femoropopliteal Bypass in Critical Leg Ischemia Related Lower Extremity Arterial Disease With and Without Diabetes Mellitus, in the city of Rio de Janeiro, Brazil, 1996-9

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Background and Aims: Patients most seriously affected with Lower Extremity Arterial Disease (LEAD) and Diabetes Mellitus (DM), have critical ischemia that endanger the viability of lower extremity and include subject undergoing surgical revascularization procedures or limbs amputations. **Aims:** To determine the frequency of Femoropopliteal Bypass (FPBP) performed on patients with LEAD or DM critical leg ischemia related to age, gender, hospitalization length of stay (LOS), mortality and governmental disbursement, compared to amputations ones.

Methods: Data were collected from the Brazilian Public Health Care System (BPHCS), in Rio de Janeiro municipality. This system comprised 81% of health care assistance, and FPBP came from 9 public hospitals units. ICD-9 and ICD-10, were used to identify the intersections between surgical procedures. Lower Extremity Amputations (LEAs) registered were 2,132 (43.6%) cases with LEAD and DM in the same period. There were excluded 2,753 (56.3%) LEAS done to trauma, and others causes. The average hospitalization LOS was calculated yearly for LEAD and DM discharges. Per-operative LEAD and DM mortality were analyzed yearly. Governmental disbursement includes hospital stay, referred to a prefixed FPBP and amputations values.

Results: Data from BPHCS registered 1,108 FPBP, being 341 (32.5%) related to LEAD (in 48 with DM and 293 without DM). Mean age was SD 9.1 y (Median 67 y) for LEAD with DM and 68 SD 10.5 y. Median 68 y) without DM (p<0.001). The male / female ratio was 1.18 for LEAD with DM, and 1.90 without DM (p<0.001). The average of hospitalization LOS was: LEAD with DM 19 SD 13.4 days (range: 3 to 102), and without DM 20 SD 13.5 days (range 1 to 385). In-hospital mortality were not registered for DM, while without DM was 8.64%. The average disbursement for the total and for each patients/y., was, respectively: with DM, US\$ 9,836.00 / y., and US\$ 819.00 / y.; without DM, US\$ 80,041.00 / y., and US\$ 1,096.00 / y. The number of amputations was 533 / y., being 341 (63.9%) with DM, and 192 (36.1%) without DM. The average amputations disbursement was: DM, US\$ 220,831.00 / y., and 647.00 / y., and without DM US\$ 124,224.00 / y., and 647.00 / y.

Conclusions: FPBP has to be improved as an obvious advantage of preserving limbs. The average disbursement was 22.5 folds higher to amputate LEAD with DM, while without DM was of 1.5. Non-traumatic amputations are an important and costly problem. Systematic approaches to reduce its burden are needed.

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Major Lower Extremity Amputations Related to Diabetes Mellitus and Peripheral Arterial Disease in the city of Rio de Janeiro, Brazil, in the period 1990-2000.

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Background and Aims: To estimate incidence rates of Major Lower Extremity Amputations (LEAs) related to Diabetes Mellitus (DM) and Peripheral Arterial Disease (PAD), their amputation level and age distribution.

Methods: Amputation data came from Amputation Register (AR) corresponding to 43 Hospital Units. The population were 4,419,147 inab. aged 30-89 y. and 1,080,141 aged 55-74 y. Prevalence rates for DM and symptomatic PAD were based on information from the Brazilian Diabetes Census (9.2% for the age 30-69 y.) and published population-based studies (17% for the age-group 55-74 y.), respectively. Data from the literature indicated that 23.5% of PAD patients, had also diagnosed diabetes. The chi-square test was used to compare proportions.

Results: From the AR there were 5,339 LEAs and 5,023 (90.7%) of them were related to DM and PAD. Patients <29 and >90 y. were excluded, resulting 4,818 amputees for patients aged 30-89 y. DM accounted for 45.9%, and PAD 54%. Mean age was 64.9 SD 10.3 y. (Median 65 y.) for DM and 66.4 SD 11.9 y. (Median 66 y.) for PAD. p<0.001. The male / female ratio was 1.12 for DM and 1.61 for PAD. p<0.001. DM related first amputation level was: Above Knee (AK) 66.1%, Below Knee (BK) 28.2%, Foot 5.6%, and Hip 0.04%, and for PAD, AK 76.2%, BK 20%, Foot 3.7% and Hip 0.03%. There were no statistical difference on limb side. Bilateral first procedure were reported as 2% for DM and 3.7% for PAD. Reamputation-related to DM was 1.61% and 1.5% for PAD. In the population aged 55-74 y., the estimated incidence rates for LEAs were: DM 11.8/100,000/y., (CI95% 11.7-11.8); or 78.6/100,000 diabetics/y. (CI 78.1-79.0); PAD 13.3/100,000/y. (CI 13.3-13.4); or 78.4/100,000 PAD patients/y., (CI78.2-78.6); PAD with DM 334.3/100,000 PAD with DM patients/y., (CI335.2-333.5). The estimated incidence of LEAs related to DM in individuals aged 30-54 y., was of 20.1/100,000 diabetics/y., (CI 19.8-20.3).

Conclusions: Amputation level AK/BK either for DM and PAD, was extremely high as the first surgical procedure. Incidence of LEAs increased with age in DM and PAD patients, being 25 folds higher than in the general population when PAD was associated with DM. These results stressed the requirement of appropriate health care to DM and PAD.

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CHARACTERISTICS AND LONG-TERM FOOT SEQUELAE OF PATIENTS WITH A DIABETIC FOOT ULCER

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Background and aims: Although literature is scarce on risk profile and complications of patients with diabetic foot ulcers, these patients are considered to be at high risk for subsequent ulceration and amputation. The aim of this study is to evaluate the characteristics and risk profile of further diabetic foot complications of people with a diabetic foot ulcer.

Materials and methods: The study is set up as a 29-month prospective case-control study of 81 cases with an ulcer at enrollment and 152 controls without ulcer. 20 Possible local and systemic risk factors were analyzed and sequelae of ulcers were documented. In case an ulcer occurred standard wound care protocols were used that included off loading of ulcers, wound debridement and infection control.

Results: Plantar toe ulcers were most likely to heal during the follow-up period (81%). Dorsal toe ulcers were most likely to end in an amputation (27.6%), while patients with a submetatarsal ulcer were most likely to get an amputation that was not a direct result of the index ulcer (31.6%). Ulceration in the follow-up period occurred in 60.5% of cases and in 10.5% controls (p<0.001, odds ratio 13.0 (95% confidence interval 6.6-25.6)). Amputations occurred in 33.3% and 1.3% of the cases and controls, respectively (p<0.001, OR 37.0 (8.6-166.7)). Patients with plantar toe ulcers seemed to develop significantly more ulcers in the follow-up period (17/21 compared to 32/49, p = 0.037). Reulceration of a plantar toe ulcer was quite common (52.9%), especially compared to a reulceration of a dorsal toe ulcer (7.7%). Logistic regression analysis suggested that of the documented risk factors, only presence of an index ulceration and alcohol abuse are predictive for the development of foot ulcers.

Conclusions: Patients with a diabetic foot ulcer have more comorbidity and a much higher chance of getting another ulceration or an amputation. Ulcers at different locations have different outcome patterns. Presence of a foot ulcer and alcohol abuse are important indicators for future ulcers.

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Relaps of diabetic foot lesions in a rural outpatient clinic

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Background and aims:

The aim of the study was to describe localisation of relapsing lesions and to investigate predictive factors for recurrence in a group of patients with diabetes mellitus type 1 and 2 at high risk for amputations.

Materials and methods:

58 patients (46% women, 53.5% men, mean age 64 years (range 33 to 87 years old), mean duration of diabetes 19.5 years, type 1 6.9%, type 2 89.7%, non 1/non 2 3.5%, HbA1c 7.6%) with advanced vascular disease were included. These patients had developed ulcers of different Wagner grade (1: 46.5%, 2: 27.6%, 3: 24.1%, 4: 1.7%) which were treated by a structured program of a specialised Diabetes Foot Centre. After healing of the primary ulcer, all patients received adequate footwear and had regular footcare. During a mean observation period of 12 months, all patients were screened for recurrence lesions and their localisation. Patients with relapses (n = 41, 70.7%) and without relapse (n = 17, 29.3%) were investigated retrospectively for variables predicting recurrence using a multivariate logistic regression analysis.

Results:

Localisation of the relapses were as follows: toes 58.9%, plantar 24.8%, heel 8.6%, dorsum or edge of the foot or area of amputation 7.8%. Multivariate analysis revealed peripheral arterial disease as a significant risk factor for relapses (OR 6.9, 95% CI: 1.3 – 37.6, P < 0.025). Age, duration of diabetes, glycaemic control, hemodialysis and lasertherapy were not predictive.

Conclusions:

1.) Besides a variety of localisations, toes should be of special emphasis when examining high risk diabetic feet.
2.) Results provide empirical evidence for peripheral arterial disease being a risk factor for relapses of foot lesions in diabetic patients with high risk for amputations. Further studies are necessary to establish, whether early intervention of the peripheral artery disease diminishes the risk for recurrence.

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NEUROISCHEMIC FOOT ULCERS HAVE A 4 -FOLD INCREASED HEALING TIME COMPARE TO NEUROPATHIC FOOT ULCERS IN DIABETES.

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Background and Aims: 95% of patients referred to the foot clinic at the Karolinska Hospital have peripheral neuropathy while 60% have neuroischemic foot ulcers. The aim of this study was to investigate healing time in neuroischemic compared to neuropathic foot ulcers.

Material and Methods: A retrospective study of 68 out of 141 patients referred to the foot clinic in 1999 due to chronic foot ulcers and who healed their foot ulcers. They were studied regarding sex, age, type of diabetes, kind of ulcers and healing time. The treatment of the chronic foot ulcer included improved metabolic control with insulin treatment, infection control, non-weight bearing treatment, improvement of microcirculation with low molecular heparin and/or treatment of peripheral oedema. None of the patient needed vascular surgery.

Results: 68 patients healed their foot ulcers during 1999 with a medium healing time of 27 weeks. 18 had type 1 diabetes of whom 67% had neuroischemic ulcers and 50 had type 2 diabetes of whom 52% had neuroischemic ulcers. The median age for the type 1 diabetic patients were 65 years compared to 75 years for type 2 diabetic patients. All patients had peripheral neuropathy while 56% had macroangiopathy with neuroischemic ulcers. The medium healing time for neuroischemic ulcers were 51 weeks compared to 13 weeks for those with neuropathy.

Conclusions: Neuroischemic foot ulcers in diabetic patients can heal but with severely prolonged healing time compared to neuropathic ulcers. Thus clinical trials with local treatment of the foot ulcer may take in consideration the expected healing time of the chronic wounds in relation to their characteristics.

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DIABETIC FOOT: HOW DO WE TREAT THEM? EXPERIENCE OF A DIABETIC FOOT CLINIC

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Background and Aims: Several studies have proven that amputation rate can be reduced by more than 50% if strategies like prevention, early diagnosis and multidisciplinary approach were implemented. The distinction between neuropathic and ischaemic foot allowed us to reduce drastically the major amputations between 1985 and 1987, when our Diabetic Foot Clinic began its activity. Since then and until 1995 there were no further reductions and the rate of major amputations had stabilized around 8,2%. The aim of our study was to analyse if there was any change between 1998 and 2000.

Materials and Methods: A retrospective study was performed reviewing the clinical files of 843 new patients (327 first arrived in 1998, 260 in 1999 and 256 in 2000).

Results: Among these patients, 593 had a foot ulcer with or without infection. There were 359 patients (60,5%) with neuropathic ulcer and 234 (39,5%) with ischaemic ulcer. Characteristics of neuropathic foot patients: 51,2% males, 88,7% type 2 Diabetes; overall mean duration of Diabetes 13,4±/9.6 years. Characteristics of ischaemic foot patients: 49,2% males, 96,6% type 2 Diabetes, overall mean duration of Diabetes 16,6±/9.8 years. Patients with neuropathic or ischaemic foot, presented us with a superficial ulcer or infection (68,9% vs 63,8%), deep infection involving tendon, muscle or bone (14,3% vs 10,3%), interdigital ulceration spreading into the plantar surface (2,2% vs 2,1%), ulcer of the leg (4,5% vs 1,7%) or necrosis (10% vs 22,1%). In patients with neuropathic ulcer, we performed minor amputation in 11,6% (n=42) and major amputation in 2,5% (n=8). Twenty two patients (9,4%) with ischaemic foot were treated with minor amputation and 23 (9,4%) with major amputation. Overall, 31 of the 593 patients (5,2%) with ulcer or infection were treated with major amputation. There were no significant differences in the rate of major amputations between 1998 (5,7%), 1999 (4,6%) and 2000 (5%). However we found statistical significance between the major amputations outcome and the two types of foot (p<0,001). Necrosis showed to carry a poor prognosis (30,7% ischaemic vs 8,3% neuropathic, p=0,024). There was no further statistical significance for age, sex, type and duration of Diabetes as risk factors for major amputation.

Conclusions: This retrospective study has showed a stable number of major amputations in an acceptable range. Poor prognosis was related to necrosis and ischaemic foot. Further improvement requires harder investment in prevention at the primary health care.

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Risk of Pressure Damage to Diabetic Feet when Wearing New Footwear.

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Background and Aims: For the diabetic foot is typically reduces moisture in the skin and the body temperature of the foot. So-called "cold, dry foot" significantly contributes to the occurrence of complications related to wearing newly purchased footwear. The speed and degree of deformation of the leather from which the footwear uppers are constructed, are influenced most effectively by temperature and moisture. The purpose of this experiment was to determine whether there are significant differences in the speed of dimensional changes of footwear uppers at a group of diabetics and at a group of healthy subjects.

Materials and Methods: Special serially produced prophylactic footwear for diabetics was used. The first group was comprised of 12 type II diabetics with diagnoses of neuropathic feet, average age 63.2 years (53 to 68). The second consisted of 12 non-diabetics, average age 45.5 years (38 to 65). An internal cast was completed for the footwear, which was measured after removal from the shoe. After approximately 15 to 20 hours of wear, the subjects returned the shoes to conduct another cast.

Results: For the group of healthy subjects, the internal circumference of the footwear increased at the metatarsalphalangeal joint after approximately 70-80 hours of wear. On average, the internal circumference in this group increased by 7-8 mm, which is 2.5% of the total internal circumference. In the calculation of currently used footwear width grades, this equals 1 to 2 widths. For the group of diabetics permanent dimensional change in footwear uppers occurred after 140-160 hours of wear, which means that diabetics need twice as long a period for shaping the footwear to individual shape than non-diabetics. The internal circumference changed by only 4-5 mm, which is 1.5 % and that is also less than 1 footwear width.

Conclusions: The period of wear for new footwear is much more critical for diabetics than for the healthy population. The period of increased local pressure of footwear on the foot is extended. This situation is much more serious because pressure discomfort is not felt very intensely by diabetics.

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Offloading the Diabetic Foot Wound Utilizing the Scotchcast Boot

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Background and Aims: To evaluate outcomes associated with treatment of diabetic foot ulcers using the Scotchcast boot.

Materials and Methods: We abstracted data from the records of 180 patients with diabetes, 83.3% male with a mean age of 55.3 ± 10.9 years undergoing treatment for non-infected, non-ischemic neuropathic diabetic foot wounds at a university teaching hospital's tertiary care outpatient clinic. All patients were treated with the Scotchcast boot as the sole form of pressure relief. The average follow up for each patient was 85.9 ± 30.6 months (range 34.2-147.7).

Results: The mean time to healing for all patients was 130.5 ± 106.7 days. While there was not a significant difference in healing time between Grade 1 and 2 wounds ($p = 0.7$), superficial (Grade 1) wounds healed significantly faster than deep (Grade 3) wounds (111.5 ± 98.2 vs. 180.8 ± 138.8 days, $p = 0.01$). A total of 80% of wounds healed during the long follow up period. Of the 20% that did not heal with Scotchcast boot therapy, 10 went on to more proximal amputation (5.6% of total population), 2 required surgical intervention to heal their wounds (0.6% of total population), one patient died with an unhealed wound (0.6% of total population), and 23 patients (12.8% of total population) were lost to follow up. During the mean 7.2-year follow up period, 47 patients died (26.1% of total population).

Conclusions: The results of this study detail outcomes of patients undergoing treatment with the Scotchcast boot. This removable modality may be useful in outpatient care of deeper or complex wounds that require frequent inspection, but treatment of superficial wounds may be better suited for a device that ensures compliance.

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Long time antibiotic treatment for osteomyelitis complicating ulcers of the forefoot in diabetic patients with peripheral neuropathy.

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Background: There is continuing controversy over the management of osteomyelitis in diabetic patients with foot ulcers.

Aim: To verify whether conservative treatment with long lasting antibiotic therapy alone or associated to bone resection in the patients with exposed bone is useful to achieve healing of the ulcer and to verify whether difference can be recorded in respect to the healing rate and time to healing achieved with the antibiotic treatment associated or not to surgical approach.

Methods: We reviewed the outcome of 35 patients that in the last two years were followed in our foot clinic for neuropathic ulcers of the forefoot complicated by osteomyelitis.

Between them 20 (mean age 65 ± 12) (group A) underwent to the resection of the exposed bone, in the remaining 15 (mean age 59.1 ± 8.7) (group B) no surgical approach was addressed to remove the osteomyelitic bone. Both groups were treated with a long term antibiotic therapy mainly clindamycin and/or ciprofloxacin till the healing of the ulcer. Mean duration of diabetes was 21 ± 11 years for group A and 16.6 ± 7.4 years for group B.

Results: Healing was achieved in 12/15 (80%) of the patients of group B and 19/20 (94.8%) of the patients of group A. The time of healing was 3.92 ± 3.1 months in group A and 3.3 ± 3.2 months in the healed patients of group B. Patients of both groups with non-healed ulcer had a shorter follow-up (2.6 ± 1.2) months.

Conclusion: no differences between the groups were recorded in time of healing rate and time to healing. In the management of osteomyelitis complicating forefoot neuropathic ulcers the surgical removal of the infected bone does not reduce the time of healing. A conservative management with long lasting antibiotic therapy may be a good option to treat osteomyelitis of the forefoot.

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Validation of the S(AD)SAD system of classification of foot ulcers.

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The S(AD)SAD system of classifying diabetic foot ulcers attempts to combine precision with simplicity. Its objective is to enable identification of groups of similar lesions for the purpose of prospective research. It is called the S(AD)SAD system because it is based on the grading of five key clinical features: Size (Area and Depth), Sepsis, Arteropathy and Denervation. Each of these is graded 0-3, with 3 being the most severe. Although easy to apply, validation is required and the purpose of this study was to seek associations between classification at first referral and outcome.

495 new ulcers were registered between 1/1/2000 and 31/3/2001 and of these 280 had true outcomes during this time (healed $n=228$, amputated $n=25$, death $n=27$). Correlations were found between outcome and the five key features of baseline classification (Area $p<0.001$, Depth $p<0.001$, Sepsis $p=0.05$, Arteropathy $p<0.001$ and Denervation $p<0.05$, Spearman rho). There was also correlation between outcome and total S(AD)SAD score ($p<0.001$).

There were 389 new ulcers classified between 1/1/2000 and 31/12/2000 and similar correlations were sought between baseline classification and outcome at three months, (healed $n=129$, unhealed $n=231$, amputated $n=19$, death $n=10$). Correlations were observed between outcome type and Area ($p<0.001$), Depth ($p<0.001$), Arteropathy ($p<0.001$) and Total S(AD) SAD score ($p<0.001$), but not with either denervation or sepsis. It has been demonstrated that the healed group of ulcers are significantly different from the other ulcers in this population, $U=160$ $p<0.001$. It has also been verified using Kappa that no two variables are measuring the same aspect of the ulcer ($p<0.001$).

These and further analyses have established a close association between classification and outcome, suggesting that such a system can be used to identify separate groups of ulcers with roughly similar prognosis. This will enable a basis for randomised controlled evaluation of different interventions.

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Retinopathy: Clinical

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AN ASSOCIATION OF ELASTIN IgA ANTIBODIES WITH THE DEVELOPMENT OF RETINOPATHY IN DIABETIC CHILDREN

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Background and Aims: One important factor in the development of vascular wall alterations is degradation of the elastic fiber major protein – elastin. Elastin peptides derived from this degradation are present in the circulating blood and they are a stimulus for pathological increased production of elastin antibodies. The aim of the present study was to examine the possible association between serum elastin antibodies and the development of diabetic microvascular complications. **Materials and Methods:** Levels of elastin antibodies (IgG, IgM and IgA) were determined by ELISA in sera of 28 children with Type 1 (insulin-dependent) diabetes mellitus (mean age 11.6 ± 2.8 years, diabetes duration – 5.1 ± 2.5 years). None of the children had clinical or laboratory evidence of vascular complications. The children were followed over a period of 6 years, and 24 healthy children of similar age and sex served as a control group. **Results:** During the study, 3 diabetics developed retinopathy, 6 microalbuminuria and 2 both retinopathy and microalbuminuria. The elastin IgG antibodies showed correlation with diabetes duration ($r=0.48$, $p=0.0007$), HbA1c ($r=0.28$, $p=0.05$), triglycerides ($r=0.28$, $p=0.05$) and antibodies to advanced glycation endproducts (AGE) ($r=0.41$, $p=0.005$). Elastin IgM antibodies correlated with HbA1c ($r=0.26$, $p=0.038$) and IgA with retinopathy ($r=0.32$, $p=0.017$). **Conclusions:** Our results suggest an association between the level of elastin IgA antibodies and the development of diabetic retinopathy.

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Microvascular complications in Bangladeshi Type-2 Diabetic Individuals: BIRDEM Diabetes Care and Complication Study (DCCS).

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Background and Aims: In South-East Asia, only a few small studies have been done to evaluate the long term complications of type-2 diabetes (DM). Diabetes Care and Complication Study (DCCS) is an ongoing study, designed to evaluate the diabetes care delivery and long-term complications of type-2 diabetes in Bangladeshi subjects.

Materials and Methods: Total 1674 type-2 diabetic patients (M=791, F=883) of mean (\pm SD) age 51.5 ± 10.39 yr. were randomly selected from the out-patient department of the BIRDEM Institute of the Diabetic Association of Bangladesh. Clinical examination, glycaemic & lipid status, funduscopy, fundal photography, urinary albumin to creatinine ratio, renal function, monofilament test and ECG were done for all patients. Mean (SD) duration of DM was 7.0 ± 5.27 yr.

Results: Prevalence of hypertension (HTN) was 51.0% and that of obesity (BMI ≥ 25) 37.2%. Only 35% had HbA1c $\leq 7\%$. Dyslipidaemia was found in 71.7% subjects: 32.1% had isolated hypertriglyceridaemia (>150 mg%), 9.9% isolated hypercholesterolaemia (>200 mg%) & 19.8% had combined hyper-triglyceridaemia & hypercholesterolaemia. Prevalence of diabetic retinopathy, nephropathy & neuropathy were 28.3%, 22.0% & 17.6% respectively. All these three microvascular complications were significantly related to longer duration of diabetes ($p<0.001$ for all), poor glycaemic control ($p<0.02$, for all) & higher blood pressure (0.014 for all). Retinopathy was more frequent among the poor socioeconomic class ($p=0.001$) and those with BMI <25 ($p=0.014$). Logistic regression suggested that reduction of HbA1c by 1% might be associated with 31% risk reduction for retinopathy, 19% for nephropathy. Similarly, reduction of systolic BP by 10 mm of Hg may be associated with 19% risk reduction for retinopathy, 20% for nephropathy and 11% for neuropathy.

Conclusions: Our study revealed that among type-2 diabetic subjects every 2nd patient has hypertension, every 3rd has BMI >25 . More than every 4th patient has retinopathy, every 5th has nephropathy and every 6th has neuropathy, all of which are significantly related to poor control of glycaemia and blood pressure.

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PATHOGENESIS OF RETINOPATHY IN YOUNG DIABETIC PATIENTS IN BANGLADESH

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Background and Aims: The relative roles of duration of diabetes, glycaemic status and insulinemic status in the pathogenesis of diabetic retinopathy still remain controversial. This issue was investigated by taking advantage of the availability of a group of lean, young, normotensive, hypo- to normoinsulinemic, generally normolipidemic and insulin treated subjects in a Bangladeshi population.

Materials and Methods: Ninety-one young diabetic subjects (diabetes diagnosed under 30 years of age) were studied for endogenous insulin status by serum C-peptide (ELISA), microvascular damage by albumin-creatinine ratio or ACR (albumin by immunoturbidimetry) and retinopathy (by both funduscopy and slit lamp biomicroscopy and graded by fundus photography). Subjects were grouped as: Group A (newly diagnosed, $n=22$, age in years at diagnosis 25.2 ± 2.8 , $M \pm SD$), Group B (duration 1-4 years, $n=25$, 20.4 ± 4.1), Group C (4.1-8 years, $n=24$, 17.8 ± 5.1) and Group D (>8 years, $n=20$, 17.7 ± 4.2).

Results: All groups were found to be BMI-matched and normotensive. Group D had highest prevalence (60%) of retinopathy. Among the ACR+ subjects 95.25% had retinopathy. Percentage of retinopathy increased with the duration of diabetes (4.55% at diagnosis vs 60% at around 8 years). DR +ve group did not differ in their glycaemic status with DR -ve group as evidenced by blood glucose and HbA1C. Both ACR +ve and DR +ve groups had reduced insulin secretory capacity.

Conclusions: The data suggest the following: a) Duration of diabetes plays a central role in the development of diabetic retinopathy; b) a sudden increase in the prevalence of generalized vasculopathy and diabetic retinopathy seem to occur at around 8 years; c) almost all subjects with generalized microvascular damage seem to suffer from diabetic retinopathy; d) the insulinemic status, in comparison to glycaemic status, seems to be predominant in determining the development of diabetic retinopathy.

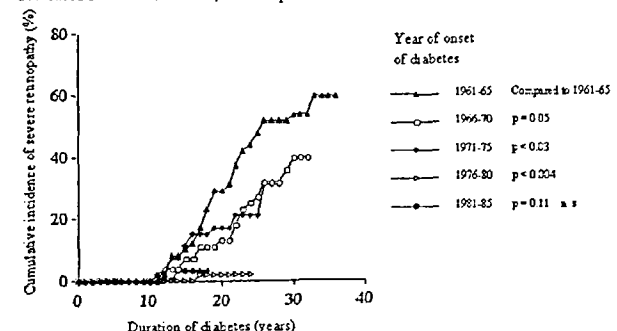
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DECLINING INCIDENCE OF SEVERE RETINOPATHY IN A POPULATION OF TYPE 1 DIABETES

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Background and Aims: In a population of Type 1 diabetes the cumulative incidence of nephropathy after 30 years of diabetes duration has decreased substantially from 32% to 12% during the past decades. We wanted to investigate if there is also a decrease of severe retinopathy. **Materials and Methods:** An historical prospective population-based long-term follow-up study. We studied all 270 patients in a district of south-east Sweden in whom diabetes was diagnosed before the age of 15 years between 1961-1985. 92% were followed until 1996-2000 or to the time of death. The rest were followed until their most recent clinic visit. Retinopathy was defined as laser treated proliferative retinopathy. Survival analysis was used. The patients were divided in five groups according to the year of diagnosis.

Results: The cumulative incidence of severe retinopathy after 25 years of duration has decreased from 44% to 25%, 21% resp. 2% for the different onset cohorts.



Conclusions: During the past decades the cumulative incidence of severe retinopathy among patients who have had diabetes for 25 years has decreased substantially.

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Predictive factors of retinopathy in normotensive, normoalbuminuric type 1 diabetic patients

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Background and Aims: To investigate factors related to the development of retinopathy in normoalbuminuric and normotensive type 1 diabetic patients.

Materials and Methods: A total of 110 normoalbuminuric and normotensive type 1 diabetic patients were included and divided into two groups based on the presence of retinopathy. Twenty four-h blood pressure measurement with readings at 20-min intervals (AMBIP) and a combination of autonomic tests based on standard, vector and spectral analysis of heart rate variation (Pro Sci Card System) were performed. Tests of heart rate variation (HRV) included the coefficient of variation (CV) and the low-frequency (LF), midfrequency (MF) and high-frequency (HF) bands of spectral analysis at rest, HRV during deep breathing (CV, mean circular resultant), Valsalva ratio and maximum/minimum 30:15 ratio. Autonomic neuropathy was characterized as an abnormality of more than one test. Fundus was graded by an experienced ophthalmologist. Urinary albumin excretion (UAE) was determined by an immunoturbidimetric assay and expressed as a geometric mean of three overnight collections. Glycosylated HbA1c was determined by immunoturbidimetry.

Results: Background or proliferative retinopathy was found in 40 patients (group 1), in the remaining 70 there were no signs of retinopathy (group 2). Duration of diabetes was significantly higher in the group with retinopathy (5.29 ± 4.9 ; 9.5 ± 5.5 respectively) ($p < 0.05$). Patients were similar regarding UAE levels, HbA1c, age, sex and physical activity. Patients with retinopathy showed significantly higher maximal night systolic and mean night diastolic blood pressure (118.95 ± 11 ; 62.95 ± 8.14) ($p = 0.04$) compared to patients without retinopathy (114.86 ± 8.91 ; 59.65 ± 7.17) ($p = 0.03$). Autonomic neuropathy was present in 22 (55%) patients with and 11 (15.7%) patients without retinopathy. Maximal night systolic BP was inversely related to CV deep breathing ($r = -0.20$, $p = 0.034$). Mean night diastolic BP was inversely related to MF ($r = -0.21$, $p = 0.032$), HF ($r = -0.25$, $p = 0.011$), CV deep breathing ($r = -0.26$, $p = 0.009$) and mean circular resultant deep breathing ($r = -0.21$, $p = 0.03$). In multiple regression analysis retinopathy was associated with the duration of diabetes ($\beta = 0.596$) and autonomic neuropathy ($\beta = 0.208$) ($p < 0.001$).

Conclusions: Duration of diabetes and autonomic neuropathy are predictive factors of retinopathy in normotensive and normoalbuminuric type 1 diabetic patients.

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Age at diabetes onset: Is it one of the determinants for diabetic retinopathy in type 2 diabetes?

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Background and Aims: To investigate whether the prevalence and severity of diabetic retinopathy (DR) differs among type 2 diabetic patients regarding to their age at diabetes onset.

Materials and Methods: This study includes type 2 diabetic patients (n:308, mean chr. age 46.9yrs) with duration of diabetes 11-15yrs (mean 13.2yrs). Patients with glaucoma, uveitis, or patients who had intraocular surgery (except vitrectomy for DR) were not included. Only the findings in the first fundus examination in our Clinic were evaluated. Diabetic retinopathy was classified as no DR, nonproliferative DR (NPDR), preproliferative DR (PPDR), proliferative DR (PDR). Patients presenting with panretinal photocoagulation scars and no proliferative changes or with no previous fundus photo or angiography were considered as panretinal photocoagulation group. Patients were grouped regarding their age at diabetes onset as 30-39yrs, 40-49yrs, 50-59yrs and 60-69yrs.

Results: The prevalence of DR in patients with duration of diabetes 11-15yrs was 59.4%. Regarding the stages of DR, NPDR was present in 41.2% of the patients, PPDR in 7.5%, PDR in 5.5% and panretinal photocoagulation in 5.2%. Regarding the age at diabetes onset the prevalence of DR was 56.5% between 30-39 yrs, 68.6% between 40-49yrs, 51.9% between 50-59yrs and 52.9% between 60-69 yrs. The prevalence of DR was higher in patients with onset of diabetes at 30-49yrs compared to 50-69yrs (64.5% and 52.0%, respectively) ($p = 0.04$).

Conclusions: Our findings show that the prevalence of diabetic retinopathy decreases in patients with age of diabetes onset at 50yrs or older. As in the lower frequency of diabetic retinopathy in patients with extensive chorioretinal atrophy, this may be explained by the hypoperfusion of retina presumably due to the arteriosclerotic changes in older ages.

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RETINOPATHY IS ASSOCIATED WITH HIGHER SERUM FREE TESTOSTERONE IN WOMEN WITH TYPE 2 DIABETES MELLITUS

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Background and aims: Adrenal and gonadal androgens may affect the incidence of macrovascular disease in both men and women. In women increased free testosterone (FT) may be accompanied by increased insulin and glucose concentrations, insulin resistance and dyslipidaemia.

Abnormalities of testosterone concentration have also been reported in diabetic patients, in women testosterone levels being higher whereas in men lower. We evaluated the gonadal profile of 74 patients with DM2 (38 men and 36 women) in association with the occurrence of retinopathy.

Materials and methods: The mean age of the patients was 63.2 ± 10.1 years. (mean \pm SEM). 54 were treated with hypoglycaemic agents and 20 with insulin. All women were postmenopausal. There was no difference between men and women in HbA1c levels, body mass index (BMI), duration of the disease and age. Variables not normally distributed were subjected to logarithmic transformation for the purpose of the statistical analysis (Mann-Whitney U test). Values are expressed as Mean \pm SEM.

Results: When data were analyzed from the whole group it was found that patients with retinopathy of any severity presented with longer duration of the disease (17.06 ± 1.52 versus 9.41 ± 1.24 years, $p < 0.001$) and higher systolic blood pressure (149 ± 4.27 versus 140.25 ± 2.40 mmHg, $p < 0.05$). In both men and women there was no difference in age, BMI, HbA1c, plasma fibrinogen, estradiol, growth hormone, IgF-1, insulin, C-peptide, thyroid hormones, sex hormone binding globulin, $\Delta 4$ -androstenedione, dehydroepiandrosterone sulphate and lipids between patients with retinopathy and those without. In men there was no association between adrenal androgens, sex hormones and retinopathy. On the contrary women with retinopathy had higher free testosterone levels (1.26 ± 0.23 versus 0.79 ± 0.14 pg/ml, $p = 0.027$).

Conclusions: It is concluded that in DM2 the occurrence of retinopathy is positively associated with the duration of the disease and systolic blood pressure. Furthermore women with retinopathy present with higher free testosterone levels, possibly suggesting a higher degree of insulin resistance.

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IS DECREASED INSULIN SENSITIVITY A SPECIFIC PREDICTOR OF PROLIFERATIVE RETINOPATHY IN TYPE 2 DIABETES?

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Background and Aims: Diabetic retinopathy (DR) is a frequent cause of visual impairment in type 2 diabetes. In this cross-sectional study we explored the relationship between DR, insulin sensitivity (IS) and other putative risk factors/markers of DR (age, BMI, diabetes duration [DUR], BP, GFR, AER, cholesterol, triglycerides and HbA1c), in 124 normotensive or treated hypertensive type 2 diabetics with different levels of AER.

Materials and Methods: IS was measured by hyperinsulinemic euglycemic clamp (insulin 80 mU/sq.m/min) and GFR by iothexol plasma clearance. DR was assessed by funduscopy and classified into 3 groups (no retinopathy [DRno, n=61], background [DRb, n=35] and proliferative retinopathy [DRp, n=28]). Methods of descriptive statistics and regression analysis were used. DUR, AER (mcg/min), HbA1c(%), HDL and triglycerides were log-transformed (ln).

Results: DRp and DRb differed from DRno for DUR, HbA1c, and AER; DRp differed from DRb and DRno for IS. IS was similar in DRb and DRno. There were significant associations between DR and AER (chi-square 10.64, $p = 0.03$) as well as between DR and IS (M-value, mg/kg/min; chi-square 11.84, $p = 0.003$). The best model to predict DR by logistic stepwise backward regression (without covariates) included age, lnDUR, lnHbA1c, lnAER (accuracy of 65.32%, $p < 0.05$). However, when the stepwise forward method discriminated DRp and DRb (but not DRb and DRno), the regression was saturated by M-value alone. The best inverse relationship to IS was with accuracy of 60.32% (score $b = -0.317$, $p = 0.016$).

Conclusions: Background and proliferative retinopathies of type 2 diabetes are associated with ageing, diabetes duration/control and albuminuria. Decreased insulin sensitivity is a specific marker of proliferative diabetic retinopathy.

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The Role of Combination Therapy in Type 2 Hypertensive Patients with Proliferative Retinopathy

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Background and Aims: It is well-documented that 50% of Type 2 diabetic patients have arterial hypertension. Presence of hypertension drastically increases the haemoflarm frequency in patients with proliferative diabetic retinopathy (PDR), whereas it is less possible to perform laserocoagulation in these subjects.

Materials and Methods: Two groups of hypertensive patients with PDR were studied.

Results: In Gr. 1 patients (n=20) conventional symptomatic antihypertensive therapy with b-blockers/Bisoprolol (Merck) or ACE-inhibitors/Perindopril (Servier) was used. Gr. 2 patients (n=20) were treated with Bisoprolol (5mg/once daily) and Perindopril (4 mg/once daily) for 6 months. At entry mean fasting blood glucose (FBG) and HbA1c levels in both groups were: FBG=130±15mg%; HbA1c=12.2±2.5%; SBP=150±50 mmHg, DBP=90±20 mmHg; total cholesterol=9.2±4.5 mmol/l, LDL=5.2±2.5 mmol/l, BMI>28. Glycemia control was achieved with Gliclazide(Servier), Glimepiride (Aventis Pharma) and Metformin (Berlin-chemie, Menarini Group), in ten case insulin (Actrapid, Insulatard, Novo Nordisk) was also used. In Gr. 1 good blood pressure control was not achieved. Fasting plasma glucose and HbA1c levels at six month were 105±10 mg% and 7.2±1.1%, respectively. Results: As a result of combination therapy with Bisoprolol (5mg) and Perindopril (4mg) blood pressure decreased to SBP=120±10 mmHg, DBP=70±10 mmHg. Frequency of haemoflarm development in Gr. 2 patients decreased by 36%, while possibility to successfully perform laserocoagulation increased by 42%, emptying of neovascular complexes after laserocoagulation was observed.

Conclusions: Combination therapy of arterial hypertension in well-controlled Type 2 diabetic patients with PDR decreases the rate of sever visual complication development in these patients.

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Automatic assessment and grading of the quality of digital retinal images for application in a telematic system supporting screening for the diabetic retinopathy

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Background and Aims: A web-based telematic system for distant telediagnosis and teleconsultation of the diabetic retinopathy was designed and developed. In the developed system, a primary care physician or a diabetologist takes a patient's retinal images with a digital fundus camera and uploads them together with additional anamnesis results to a distant database through the internet. The digital retinal images are retrieved and evaluated by an expert ophthalmologist and then annotations of lesions and diagnosis are sent back to the physician. The aim of this work was to elaborate a method for automatic assessment and grading of the quality of digital retinal images to be applied on the physician's site of the developed system.

Materials and Methods: An index describing quality of digital retinal images in terms of sharpness, contrast and visibility of fine structures was elaborated. The designed index bases on one-dimensional (1-D) averaged intensity power spectrum normalized by mean intensity and size of a digital retinal image. It is defined as average slope of the above described 1-D power spectrum over a certain frequency range adjusted by a blur-dependant factor. Ability of the developed index to differentiate between good and bad quality images was tested basing on a set of 200 color digital retinal images of diabetic as well as healthy subjects. The whole set of images was divided into 4 sub-sets with different perceived quality (bad, fair, good, excellent) as judged by the authors and values of the designed index in each sub-set were studied.

Results: The average quality index for the whole set of 200 images equals to 5.0 +/- 0.7 (Mean +/- SD). The following results were obtained in the 4 sub-groups with different perceived quality: 4.20 +/- 0.3 (bad quality), 4.60 +/- 0.3 (fair quality), 5.10 +/- 0.5 (good quality) and 5.70 +/- 0.5 (excellent quality). ANOVA confirmed high statistical significance of the obtained differences ($p < 1e-44$). Differences between all pairs of consecutive sub-groups (i.e. bad vs. fair, fair vs. good and good vs. excellent) were also statistically significant (t-test, $p < 1e-7$).

Conclusions: The developed index seems to be able to properly differentiate quality of digital retinal images. It can be applied in the developed telematic system as a supportive tool, ensuring acceptable quality of the digital retinal images taken by primary care physicians and diabetologists.

1102

HIGH RESOLUTION DIGITAL RETINAL CAMERA: DOES USE OF TWO IMAGES PER EYE INCREASE SCREENING TIME?

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Background and aims: The U.K. National Screening Committee recommends 2 overlapping digital views of each retina in screening for diabetic retinopathy. As the Newcastle service screens 8,800 patients per year the move from a single macula-centred Polaroid photograph per eye to 2 images per eye could potentially cause problems of throughput by a small increase in unit time to screen each individual. **Methods:** Since August 2000 a novel high resolution imaging system has been in use providing 1360 by 1024 pixels per image via a JVC progressive scan CCD camera. The original Polaroid system has been maintained during the change over period. The actual time taken for the photographic procedure was recorded for each system excluding the common factors of visual acuity, dilatation, ophthalmoscopy and patient feedback. **Results:** Photographic time per patient was 186±32 (SD) for Polaroid and 102±34 seconds for digital imaging ($p < 0.0001$). From the patient's perspective the five-fold lower flash intensity was much lower as assessed by linear analogue scale (4.8 ± 2.2 vs. 8.6 ± 1.3 ; $p < 0.0001$). The resolving power of the digital image meets the new stringent National Screening Committee criteria. Viewing on 15 inch monitors avoids the operator eye strain of examining Polaroid prints and facilitates the process of patient feedback. **Conclusions:** The new generation high resolution digital camera improves patient acceptability and image resolution. Despite the doubling of retinal photographs per patient, unit time for photography is markedly decreased.

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PROGRESSION OF RETINOPATHY WITH INSULIN GLARGINE OR NPH INSULIN – A MULTI-TRIAL ANALYSIS

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Background and Aims: As part of the development programme for insulin glargine (Lantus), progression of retinopathy in diabetes was assessed to provide reassurance on safety. **Materials and Methods:** In four large randomized, multicentre, open-label studies, 2207 patients received insulin glargine (Lan) or NPH insulin (NPH) for 28-52 weeks. Change in retinopathy was evaluated by ophthalmological examination, fundus photography, and reports of retinal adverse events. The findings were evaluated by a panel of four ophthalmologists and one physician, all with a special interest in diabetes. **Results:**

Finding	Type 1 diabetes				Type 2 diabetes			
	3001 (%)		3004 (%)		3002 (%)		3006 (%)	
	Lan	NPH	Lan	NPH	Lan	NPH	Lan	NPH
Progression								
Clinical exam	4.8	5.7	9.5	7.1	8.4	13.0	9.2	10.7
Photography	5.3	3.4	3.2	3.9	5.9	9.1	7.5	2.7
New PDR								
Clinical exam	1.9	1.1	1.3	1.7	0.7	0	2.7	1.3
Photography	2.2	2.6	1.8	3.8	2.1	1.8	4.1	2.2
New ME								
Clinical exam	3.7	1.9	0.9	1.3	1.8	2.4	3.1	3.0
Photography	6.9	7.9	0.9	1.3	11.2	6.5	2.8	2.2
Retinal AEs	18.0	12.0	9.8	10.4	3.1	2.5	22.0	24.7
Disc swelling	0	0	0	0	0	0	0	0

PDR: proliferative diabetic retinopathy; ME: macular oedema; AEs: adverse events
Conclusions: The comparison of fundus photography and ophthalmological examination showed little agreement between the methods. Two observed differences on photography were found to be inconsistent between methods of assessment and between studies (ME in study 3002; μ 3-step progression in study 3006). These results taken as a whole do not suggest any increased risk in the development or progression of diabetic retinopathy in patients treated with Lantus compared with NPH insulin.

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Retinopathy: Experimental/Pathogenesis

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RETINOPATHY IN MONKEYS WITH SPONTANEOUS TYPE 2 DIABETES

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Background and Aims: Type II diabetes occurs spontaneously in Rhesus monkeys and shows an extraordinary similarity to human diabetes in terms of clinical features and relative time course. The purpose of this study was to clinically and histopathologically investigate ocular vascular changes in these monkeys. Methods. Bilateral ICG and fluorescein angiograms, duplex Doppler ultrasounds of the carotid and retrobulbar circulations, ganzfeld ERGs, and ophthalmoscopic examination were performed on aged normal and diabetic monkeys. A multifocal ERG (mERG) was performed on the most severely affected of the animals. Retinas from 10 diabetic monkeys were incubated for ADPase activity (labels viable retinal blood vessels) and nonspecific esterase (label PMNs) and flat-embedded. Two micron sections were taken through areas of interest.

Results: Cotton wool spots, intraretinal hemorrhages, arteriolar narrowing, and hard lipid exudates in the macula were observed by ophthalmoscopy in some monkeys. ICG angiograms showed areas of delayed choroidal filling and/or non-perfusion in some monkeys. The Doppler measurements of posterior ciliary peak systolic velocity were significantly reduced in two monkeys. ERGs were profoundly reduced in both inner and outer retina in some animals. The multifocal ERG (mERG) recorded from a severely affected eye showed a large reduction in amplitude in the inferior retina as well as an area of reduction nasal to the macula, approximately corresponding to the areas of ICG filling abnormalities. Large nonperfused areas extending from disk to midfovea were observed in two monkeys. Other diabetic monkeys had smaller nonperfused areas. There were apparent fluid filled spaces in the outer plexiform layer in one of these maculas suggesting macular edema. Dot/blot hemorrhages, cotton wool spots, and microaneurysms were documented in retinas. The greatest number of PMNs in vascularized retina were in the posterior pole, and specifically, in the parafoveal area. The animals with the most severe diabetes had the greatest number of PMNs, and a normal control monkey, who was 15 years old, had the fewest PMNs. The most severe retinopathy was observed in diabetic monkeys with hypertension.

Conclusions: Monkeys with type II have many of the angiopathic changes associated with human diabetic retinopathy. Severity in diabetes and retinopathy was associated with increased PMNs in retina. Reductions in ERG corresponding to areas with ICG filling abnormalities suggest poor choroidal perfusion as the source of these ERG reductions.

1106

INTERCELLULAR ADHESION MOLECULE - 1 (ICAM - 1, CD 54) IN PATIENTS WITH DIABETIC RETINOPATHY (DR).

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Background and Aims: Cell adhesion molecules (CAMs) have been demonstrated to be elevated in diabetes mellitus and implicated in the course of micro- and macrovascular complications. One of the CAMs - ICAM - 1 (CD 54) is expressed by different cell types. The aim of the study was to elucidate the clinical significance of ICAM - 1 expression on leucocytes (lymphocytes and granulocytes) in Type 1 DM and DR. **Materials and Methods:** 83 patients (37 females and 46 males, age median 26.4 years old) with Type 1 DM & various stages of DR were estimated on the ICAM - 1 (CD 54) expression on blood lymphocytes and granulocytes by flow cytometry using FACScan (Becton - Dickinson) & compared with healthy controls (n = 17). **Results and Conclusions:** Table 1. ICAM - 1 (CD 54) Expression on Lymphocytes & Granulocytes in Patients With Type 1 Diabetes and Diabetic Retinopathy.

Characteristics	DR 0	DR 1	DR 2	DR 3 (active)	DR 3 (quiescent)	Healthy Controls
N of patients	17	12	9	17	5	17
HbA1c	8.0±0.2	8.1±0.3	8.3±0.3	8.6±0.5	8.5±0.4	5.1±0.3
Lymphocytes CD 54 ^a	11.8±2.9	11.05±2.07	16.13±4.8	25.13±4.8 ^b	11.50±3.5	12.41±3.02
Granulocytes CD 54 ^a	5.7±5.02	13.59±3.62	15.68±4.89	52.5±1.62 ^b	5.28±1.62 ^b	8.33±3.01

a - versus controls, DR 0, DR 1 (p < 0.01); b - versus DR 2, DR 3 active (p < 0.01)

We observe the rising CD 54 expression tendency in patients with DR 3 (active) and the low in DR 0, DR 1 & DR 3 (quiescent), reflecting the increased & decreased active and adhesive ability of the mentioned cells on these stages accordingly. There needs further investigation of this mechanism, its correlation with metabolic control & DR prognosis.

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Prevention of retinal leukostasis by gliclazide in streptozotocin-induced diabetic rats

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Background and Aims: Some sulfonylurea anti-diabetic agents might affect vascular function. However, gliclazide, a sulfonylurea, was reported recently to reduce neutrophil and monocyte adhesion to cultured endothelial cells in hyperglycemia. This study was designed quantitatively to evaluate the effectiveness of gliclazide treatment in vivo on leukostasis in the retinal microcirculation of diabetic rats.

Materials and Methods: Diabetes was induced in male Brown-Norway rats by intravenous injection of streptozotocin (65 mg/kg). Gliclazide or glibenclamide was mixed with bait and administered orally during a 3-week period of diabetes. Retinal leukostasis was quantitatively evaluated in vivo with acridine orange leukocyte fluorography using scanning laser ophthalmoscopy.

Results: The number of leukocytes trapped in the area around the optic disc (radius, 3 disc diameters) in diabetic rats (32.5±1.3 cells; n=4) was significantly increased compared with nondiabetic control rats (20.3±3.4 cells; n=4) (P<0.05). However, gliclazide-treatment significantly reversed the number of leukocytes trapped in the retina (22.0±5.3 cells; n=4) (P<0.05). In contrast, no significant reduction in retinal leukostasis was found in the glibenclamide-treated diabetic rats compared with the untreated diabetic rats.

Conclusions: Treatment with gliclazide decreases leukostasis in the retinal microcirculation in early diabetes. From this observation, gliclazide might contribute to improved retinal blood flow and have a therapeutic effect on diabetic retinopathy, preventing microcirculatory disturbances caused by trapped leukocytes.

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STUDY ON THE MECHANISM OF NEUTROPHIL ADHESION TO RETINAL CAPILLARY ENDOTHELIAL CELLS UNDER HIGH GLUCOSE CONDITION

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Background and Aims: Diabetic retinopathy is a leading cause of adult vision loss and blindness. Much of the retinal damage that characterizes the disease results from retinal vascular leakage and occlusion. Capillary occlusion is the result of microvascular thrombi in which erythrocytes, platelets and leukocytes each may play a role. We investigate the pathogenesis of leukocyte stasis by exposing bovine retinal capillary endothelial cells (BRCECs) for high glucose concentration. **Materials and Methods:** We examined the adhesion of neutrophils to BRCECs incubated in media containing 5.5-30 mmol/L D-glucose for 24 hours. We also measured the expression of E-selectin on endothelial cells and the activation of NF(nuclear transcription factor)-κB in nuclear fractions of endothelial cells by using electrophoretic mobility shift assay. **Results:** We observed that 30 mmol/L D-glucose significantly increased the adhesion of neutrophils to BRCECs (12.5% vs. 3.0%, p<0.01) and migration of neutrophil across cultured BRCEC monolayers (41.0% vs. 21.0%, p<0.05) in respect to 5.5 mmol/L D-glucose. The expression of E-selectin was increased incubated with 30 mmol/L D-glucose compared with 5.5 mmol/L D-glucose (1.45 OD vs. 0.54 OD, p<0.01). Electrophoretic mobility shift assay of nuclear extracts of BRCECs exposed for 24 h to 30 mmol/L D-glucose revealed an intense NF-κB activation compared with cells cultured in 5.5 mmol/L D-glucose (8.72×10⁴ counts×mm² vs. 1.88×10⁴ counts×mm², p<0.01). **Conclusions:** These results suggest that high glucose concentration promote neutrophil adhesion to the BRCECs through upregulation of cell surface expression of E-selectin, possibly depending on NF-κB activation and may have implications for the induction of microvasculopathy of diabetic retinopathy.

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Free Insulin-like Growth Factor-I and Vascular Endothelial Growth Factor in vitreous fluid of patients with diabetic retinopathy: study of their relationship and behaviour.

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Background and Aims: The aim of the study was to investigate the relationship between free Insulin-like Growth Factor-I (IGF-I) and Vascular Endothelial Growth Factor (VEGF) in the vitreous fluid of diabetic patients with proliferative diabetic retinopathy (PDR).

Material and Methods: 37 diabetic patients in whom a vitrectomy was performed were compared to 21 non-diabetic patients with other conditions requiring vitrectomy, but in whom the retina was not directly affected by neovascularization. Statistics: Mann-Whitney U-test and the Spearman rank correlation coefficient. The results are expressed as the median and range.

Results: Vitreal levels of both free IGF-I and VEGF were higher in diabetic patients with PDR than in control subjects (0.16 ng/ml [0.06-0.47] vs. 0.11 ng/ml [0.06-0.21]; $p < 0.01$, and 1.38 ng/ml [0.11-7.61] vs. 0.009 ng/ml [0.009-0.038]; $p < 0.0001$, respectively). We also detected higher intravitreal protein concentration in diabetic patients with PDR than in control subjects (3.01 mg/ml [1.05-13.8] vs. 0.73 mg/ml [0.27-2.62]; $p < 0.0001$). After adjusting for total intravitreal protein concentration, VEGF (ng/mg of proteins) remained significantly higher in diabetic patients with PDR than in control group (0.38 [0.01-4.72] vs. 0.01 [0.003-0.033]; $p < 0.0001$). By contrast, the ratio of free IGF-I/vitreous proteins (ng/mg of proteins) was higher in control subjects than in diabetic patients (0.15 [0.06-0.65] vs. 0.06 [0.01-0.45]; $p < 0.0001$). The vitreous concentrations of VEGF were higher in patients with active PDR than in patients with quiescent PDR (2.06 ng/ml [0.22-6.66] vs. 0.57 ng/ml [0.11-1.26]; $p < 0.005$). However, vitreous free IGF-I was not related to PDR activity. VEGF was 10-fold higher in vitreous fluid than in serum from patients with PDR ($p < 0.0001$). By contrast, in the control group VEGF was higher in serum than in the vitreous fluid ($p < 0.0001$). Free IGF-I was higher in serum than in vitreous fluid in diabetic patients with PDR ($p < 0.001$), and also in control subjects ($p < 0.001$). Finally, we did not observe a correlation between the vitreal levels of free IGF-I and VEGF, neither in diabetic patients, nor in the control group.

Conclusions: We conclude that free IGF-I and VEGF behave differently and that they are not related within the vitreous fluid of diabetic patients with PDR. In addition, our results suggest that VEGF but not free IGF-I is directly involved in the pathogenesis of PDR.

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EFFECTS OF ANGIOTENSIN II AND INSULIN ON TGF- β PRODUCTION BY BOVINE RETINAL PERICYTES.

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Background and Aims: TGF- β is a cytokine involved in several cell functions, such as regulation of proliferation and turnover of extracellular matrix, inducing synthesis of type IV collagen, fibronectin, laminin and proteoglycans. TGF- β levels appear to be elevated in type 2 diabetes, particularly in retinopathy. Thickening of the basement membrane is peculiar of diabetic microangiopathy and could be mediated by increased synthesis of TGF- β . Angiotensin II (AT II) and insulin were reported to induce TGF- β production in mesangial cells. Since retinal capillary pericytes are homologous to mesangium, we hypothesized that AT II and insulin could increase TGF- β release also in pericytes. This study aimed at verifying this hypothesis in bovine retinal pericytes (BRP) in culture.

Materials and Methods: BRP were cultured in 6-well plates until subconfluent. Cells were then incubated overnight with insulin or AT II 10-10 mol/l, 10-8 mol/l, 10-6 mol/l or 10-4 mol/l. TGF- β concentrations were measured in the supernatants by ELISA and expressed as pg/ml/106 cells.

Results: AT II and insulin addition did not modify significantly TGF- β production by BRP. AT II: basal 6138.8 \pm 4281, 10-10 mol/l 3502.3 \pm 3077, 10-8 mol/l 2951.6 \pm 2804, 10-6 mol/l 3654.3 \pm 3186, 10-4 mol/l 3583 \pm 3146. Insulin: basal 4404.3 \pm 3196, 10-10 mol/l 3737.3 \pm 4879, 10-8 mol/l 3637.3 \pm 4929, 10-6 mol/l 3003 \pm 2540, 10-4 mol/l 2440 \pm 2240.

Conclusions: TGF- β production by pericytes does not appear to be stimulated by addition of AT II or insulin. This suggests that thickening of the basement membrane in retinal capillaries may not be mediated by these hormones.

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REPLICATION OF BOVINE RETINAL PERICYTES FOLLOWING INHIBITION AND ACTIVATION OF PROTEIN KINASE C.

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Background and Aims: Protein kinase C (PKC) is a family of enzymes involved in the regulation of various cell functions. Abnormal PKC activity could be involved in the pathogenesis of diabetic microangiopathy. The aim of this study was to evaluate the effect of an activator (phorbol-12-myristate-13-acetate, PMA) and an inhibitor of PKC b1- and b2-isomers (LY379196, generously provided by Ely Lilly, Indianapolis) (iPKC) in the presence of physiological (5.6 mmol/l) or high (28 mmol/l) glucose concentrations.

Materials and Methods: To study the effects of PKC inhibition, bovine retinal pericytes (BRP) were incubated with either physiological or high glucose, with or without 500 nmol/l or 1 μ mol/l iPKC. Cells were counted after 7 days and DNA synthesis through BrdU incorporation was measured after 4 days by ELISA. To study PMA effects on cell replication, BRP in normal or high glucose were exposed for 60 minutes every day to 100 or 500 nmol/l PMA. To confirm that PMA effect was due to PKC activation, BRP were stimulated by PMA, followed by iPKC 1 μ mol/l. **Results** are expressed as percentages of 5.6 mmol/l glucose. **Results:** Means \pm SD of cell counts were: 89.7 \pm 11.4% for 28 glucose ($p = 0.003$ vs 5.6 glucose), 65.1 \pm 14.4% for normal glucose + 500 nmol/l iPKC ($p = 0.000$ vs 5.6 glucose), 61 \pm 11.5% for 28 glucose + 500 nmol/l iPKC ($p = 0.001$ vs 28 glucose), 52.3 \pm 22.8% for 5.6 glucose + 1 μ mol/l iPKC ($p = 0.001$ vs 5.6 glucose), 44.9 \pm 11.5% for 28 glucose + 1 μ mol/l iPKC ($p = 0.000$ vs 28 glucose); 581.3 \pm 328% for 5.6 glucose + PMA 100 nmol/l ($p = 0.016$ vs 5.6 glucose), 696.9 \pm 276.6% for 5.6 glucose + PMA 500 ($p = 0.003$ vs 5.6 glucose), 391.1 \pm 139.2% for 28 glucose + PMA 100 ($p = 0.001$ vs 28 glucose), 566.8 \pm 272.7% for 28 glucose + PMA 500 ($p = 0.005$ vs high glucose), 310.9 \pm 100% for 5.6 glucose + PMA 500 + iPKC 1 μ mol/l ($p = 0.003$ vs 5.6 glucose), 145.2 \pm 36.2% for 28 glucose + PMA 500 + iPKC 1 μ mol/l ($p = 0.007$ vs high glucose). Absorbances of cells grown in 28 mmol/l glucose were lower than in normal glucose (83.2 \pm 15.8%, $p = 0.03$); iPKC reduced absorbances both in addition to normal (66.4 \pm 14.6%, $p = 0.003$) and high glucose (70.8 \pm 18.8%, $p = 0.02$), whilst PMA addition increased them in either condition (167.2 \pm 38.9%, $p = 0.006$ in normal glucose and 154 \pm 35.1%, $p = 0.01$ in high glucose). **Conclusions:** The addition of an activator and an inhibitor of PKC is able to influence BRP replication in the presence of physiological and high glucose. These data confirm results we obtained previously with endothelial cells and suggest that the increased activity of PKC induced by high glucose, as described by other Authors, may not be responsible for impaired cell replication in diabetic retinopathy.

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ENDOGENOUS mRNA LEVELS OF ANTIOXIDATIVE ENZYMES IN THE RETINA OF RATS WITH STZ-INDUCED DIABETES.

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Background and aims: Long-term hyperglycemia results in microvascular complications, like diabetic retinopathy, but the mechanisms behind the development of microangiopathy are not clear. Oxidative stress, either due to increased production of reactive oxygen species or a deficient endogenous defense, has been suggested. The aim of the present study was to study effects of hyperglycemia on the endogenous antioxidative enzyme systems in the retina *in vivo* and if so, whether aminoguanidine or probucol, drugs that can act as an inhibitor of advanced glycosylated end products or as a free radical scavenger, had any influence. **Materials and methods:** Male Wistar rats, body weight 250-300 g, were injected intraperitoneally, 1-2 times, with streptozocin (STZ) (60 mg/kg body weight). Only rats with blood glucose levels ≥ 15 mmol/L were included. Diabetic as well as strain matched control animals were fed either 1) a normal diet, 2) addition of probucol (1% w/w) in the pellets or 3) aminoguanidine (0.5 g/L for diabetic and 1 g/L for control rats) in the drinking water. After one and six months, respectively, the retina was snap frozen, RNA was extracted, and the mRNA levels for CuZnSOD, MnSOD, catalase and glutathione peroxidase (GSH-Px) were analysed using Quantitative Competitive Polymerase Chain Reaction (QC-PCR). **Results:** The mRNA catalase levels in the retina were low, about 1/10 of those of the other enzymes but there was a 100% increase after six months of diabetes (0.012 \pm 0.004 vs. 0.006 \pm 0.002; $p = 0.006$). Neither aminoguanidine nor probucol had any influence on this elevated concentration. There was a 50% increase of relative mRNA concentrations of GSH-Px after six months of diabetes, (0.83 \pm 0.25 vs. 0.54 \pm 0.18; $p = 0.042$), which was inhibited by probucol. **Conclusion:** The elevated retinal mRNA levels of catalase and GSH-Px after six months of diabetes, and the normalization of the GSH-Px levels by probucol, suggest that long-term exposure to hyperglycemia results in increased oxidative stress in the retina.

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Concentrations of Adrenomedullin(AM) and VEGF in the Aqueous Humor of the Anterior Chamber at Each Stages of Diabetic Retinopathy

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Background and Aims: In order to elucidate pathophysiology of diabetic retinopathy from the standpoint of microcirculatory hemodynamics, the roles of AM and VEGF in the onset and progress of retinopathy were studied at each stage of retinopathy.

Materials and Methods: Enrolled in the study were 32 diabetic patients and 28 non-diabetic patients, that is, the control group. Aqueous humor was collected during ophthalmologic operations excluding that of neovascular glaucoma, and AM was measured by RIA method and VEGF by EIA method. Concentrations of AM and VEGF were compared according to duration of diabetes mellitus, HbA1c and stages of retinopathy.

Results: AM concentration(mean±SD fmol/ml) in the aqueous humor of the anterior chamber also seemed to increase as duration of diabetes mellitus lengthened. VEGF concentration(mean±SD pg/ml) in the aqueous humor of the anterior chamber was 217.929±254.734 in the diabetic group, or a significant increase($p<0.01$) in the diabetic group.

Conclusions: AM concentration in the pre-proliferation group was significantly higher than that in the simple group($p<0.01$) and the highest among three groups. Increases in AM concentration in the aqueous humor of the anterior chamber in the simple and pre-proliferation retinopathy are thought to substantiate the increase in retinal blood flow at these stages. It is also inferred that the decrease in blood flow at the proliferation stage accompanies the decrease in AM. The distribution dynamics are similar to changes in blood concentrations of AM, suggesting the possibility of the association between the increase in VEGF and the change.

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INHIBITION OF ADVANCED GLYCATION/LIPOXIDATION ENDPRODUCTS (AGES/ALES) PREVENTS RETINOPATHY IN EXPERIMENTAL DIABETES

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Background and Aims: Pyridoxamine (PM) is a new AGE/ALE inhibitor that traps carbonyl intermediates in advanced glycation and lipoxidation reactions. We examined the ability of PM to protect against diabetic retinal disease and compared it with the antioxidant vitamin E (VE) known to prevent retinopathic lesions in diabetic rats.

Materials and Methods: Streptozotocin (STZ) was used to induce diabetes in Sprague-Dawley rats and after one week the animals were treated with either PM (1g/l drinking water) or VE (2000 IU/Kg diet). Glycated haemoglobin was monitored throughout and maintained at ~12% with injection of insulin (3IU, 3 times/ week). After 29 weeks of diabetes the animals were sacrificed and a range of AGEs / ALEs were measured in skin collagen. The eyes from each animal were removed and the retinas examined for retinopathic change. Retinas from at least 8 animals per group were subjected to trypsin digestion and the number of acellular capillaries estimated. The retinas from the fellow eyes (n=8/group) were extracted for total RNA, cDNA produced by reverse transcriptase and quantitative real-time PCR conducted to determine regulation of extracellular matrix (ECM) mRNAs.

Results: The results demonstrated a >3 fold increase in acellular capillaries and significant upregulation of fibronectin (2 fold) and laminin (b chain) (2.6 fold) mRNA expression when diabetic controls were compared to non-diabetics. PM treatment resulted in considerable protection against capillary drop-out ($p<0.01$) and a significant reduction in ECM mRNA expression over diabetic controls; fibronectin ($p<0.02$), laminin ($p<0.001$). VE treatment of diabetics did not cause statistically significant protection from retinal capillary death nor was fibronectin expression altered although there was a reduction in diabetes-related laminin mRNA-induction ($p<0.01$). The retinal data also correlated with AGE/ALE levels in other non-ocular tissues with PM lowering levels more effectively than VE.

Conclusions: The results suggest that AGE/ALE-inhibition during experimental diabetes using PM can prevent several of the recognised parameters of diabetic retinopathy. While the antioxidant VE had a less significant protective effect than PM, it would appear that hyperglycaemia-related advanced lipoxidation and advanced glycation processes may play a role in retinal vascular dysfunction during diabetes.

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IS HOMOCYSTEINEMIA A MARKER OF DIABETIC RETINOPATHY IN HYPERTENSIVE TYPE 2 DIABETICS?I. Iliev¹, A. Parvanova¹, J. Zalete¹, B. D. Dimitrov^{1,2}, D. Fenili³, A. Perna¹ and P. Ruggerenti¹

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Background and Aims: Fasting hyperhomocysteinemia is a marker of cardiovascular disease. In this cross-sectional pilot study we tested whether homocysteinemia (HCY, $\mu\text{mol/l}$) is an independent marker of retinopathy (DR) in hypertensive type 2 diabetics.

Materials and Methods: 37 consecutive patients with median (range) GFR 88.5 (32-137 ml/min/1.73 sq.m.) and AER 69 (2.4-1609 mcg/min) were assessed by fundoscopy for DR. GFR was measured by iothexol plasma clearance. Methods of descriptive statistics and logistic regression were used. HCY, diabetes duration (DUR, months) and AER were logarithmically transformed to correct for skewed distribution and/or outliers.

Results: The patients with DR (DRy, n=11) and without DR (DRn, n=26) were homogeneous for age (mean 60 years, SD 7 years), HbA1c (6.2%, 1.5%) and serum lipids. The patients with DR had higher lnHCY, lower BMI (kg/m^2), longer lnDUR, higher systolic BP (SBP) and higher lnAER (see mean and SD in Table, $p<0.05$). In the stepwise conditional logistic regression, as being partially independent, lnHCY (score b=4.5) together with lnDUR (b=2.1) and SBP (b=0.1) predicted DR with accuracy of 86.5% ($p<0.05$). The study was underpowered to confirm GFR as a likely marker.

Retinopathy	lnHCY	BMI	lnDUR	SBP	lnAER
DRy	2.8(0.4)	27.4(5.1)	4.9(0.8)	155(15)	5.1(1.4)
DRn	2.5(0.3)	31.3(5.3)	3.9(0.9)	145(12)	3.8(2.0)

Conclusions: Fasting homocysteinemia is a marker of diabetic retinopathy in hypertensive type 2 diabetics, additionally to diabetes duration and systolic blood pressure, and might be used as likely predictor of its progression.

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Autonomic Neuropathy

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RELATIONSHIP BETWEEN IMPAIRED 0.1 HZ VASOMOTION AND PARASYMPATHETIC AND SENSORY NEUROPATHY IN DIABETES

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Background and Aims: In diabetic patients a reduction of 0.1 Hz skin blood flow fluctuations was found, which points to a decreased efferent sympathetic activity to the skin. We investigated the relationship between impaired 0.1 Hz vasomotion and parasympathetic and sensory neuropathy. **Materials and Methods:** 20 type 1 and 19 type 2 diabetic patients were investigated. Vasomotion was recorded in single capillaries at the dorsal middle phalangeal area of the left ring finger by means of laser Doppler anemometry. Parasympathetic neuropathy was assessed by spectral analysis of heart rate variation during rest and recording heart rate responses to deep breathing and Valsalva manoeuvre. Sensory neuropathy was investigated by measuring pain, vibration and thermal sensory thresholds. **Results:** Loss of spontaneous variations in skin blood flow was found in 48.7% of all patients. Vasomotion (0.09-0.1 Hz) was more frequently ($p<0.05$) impaired in diabetic patients, types 1 and 2, with at least one altered test for parasympathetic neuropathy (81.3%), but it was already reduced in those with normal heart rate variability (47.4%). Especially in type 1 diabetic patients loss of spontaneous fluctuations in skin blood flow was found in all persons (100%) with parasympathetic neuropathy but even in 41.7 % of those with normal tests for cardiac autonomic neuropathy ($p=0.01$). The prevalence of impaired vasomotion was higher in type 2 diabetic patients with an abnormal cold threshold compared to those with normal cold sensation (66.7% vs. 14.3%; $p<0.05$). Impairments of vibration sensation and vasomotion were not related. An abnormal warm thermal threshold was only found in 2 patients. No one had a reduced pain sensation. **Conclusions:** These results indicate that reduction of 0.1 Hz microcirculatory fluctuations as evidence of sympathetic dysfunction occurs in parallel with the parasympathetic diabetic neuropathy and impairment of cold sensation. Thus, reduction of vasomotion, detected by laser Doppler anemometry, might be an early index of autonomic dysfunction and precedes abnormalities in warm and pain sensation.

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CONTRIBUTION OF DIABETIC AUTONOMIC NEUROPATHY TO HYPOGLYCEMIA UNAWARENESS IN LONG-TERM TYPE 1 DIABETES

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Background and Aims: Iatrogenic hypoglycemia is an established, but reversible, risk factor for impaired glucose counterregulation and hypoglycemia unawareness in type 1 diabetes. Diabetic autonomic neuropathy (DAN) may cause irreversible damage to glucose counterregulation, but its contribution has not been evaluated in a large group of patients. We conducted a cross-sectional study in long-term (>10 years) diabetic patients to assess the role of DAN in hypoglycemia unawareness. **Materials and Methods:** 75 Consecutive patients (mean age \pm SD, 44 ± 13 y) with type 1 diabetes of 25 ± 11 years duration were included. Hypoglycemia unawareness was assessed using structured questionnaires and scored between 0 (maximal aware) and 10 (maximal unaware). The presence of DAN was based on standard cardiovascular reflex tests, and scored as being normal, pure parasympathetic lesion (DAN-p), pure sympathetic lesion (DAN-s), or combined lesion (DAN-p+s). HbA1c was measured using HPLC. **Results:** 59 Patients (78%) scored at least 1 point on the unawareness scale and 21 (29%) had 3 or more points, indicating moderate to severe hypoglycemia unawareness. Hypoglycemia unawareness was related to HbA1c ($r=-0.293$, $P=0.01$) and diabetes duration ($r=0.215$, $P=0.06$). As many as 52 patients (69%) had abnormalities in cardiovascular reflex tests: 8 DAN-p (11%), 14 DAN-s (18%), 30 DAN-p+s (40%). No relation was found between hypoglycemia unawareness and DAN for the whole group. Subgroup analysis showed a trend towards higher unawareness-scores in poorly controlled (HbA1c $>8.3\%$) patients with DAN-s ($P=0.096$), and lower scores (i.e. more aware of hypoglycemic symptoms) in patients with >20 years diabetes and DAN-p ($P=0.04$). **Conclusions:** In this group of long-term diabetic patients, hypoglycemia unawareness is fairly prevalent and still mainly determined by HbA1c and diabetes duration. The contribution of DAN to hypoglycemia unawareness for the entire group was small, but DAN-s may become relevant in poorly controlled patients. Remarkably, DAN-p appears to be related to increased awareness of hypoglycemia.

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NEUROPATHY STATUS, GASTRIC EMPTYING AND DIGESTIVE SYMPTOMS IN TYPE-1 DIABETES MELLITUS: IS THERE A RELATIONSHIP?

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Background and aims: the etiology of the motility disorders and the nature of the associated complaints in diabetes mellitus (DM) are still poorly characterized. The aim of this study was to determine the correlations between the neuropathy status, gastric emptying and the severity of digestive symptoms in long-standing type-1 DM. **Materials and methods:** 23 patients with type-1 DM of long duration (duration of DM: 26.1 ± 2.3 yrs, age: 45.2 ± 2.7 yrs, mean \pm SE) were included. The stomach function was evaluated by a scintigraphic gastric emptying procedure. Autonomic neuropathy (AN) was assessed by means of the five standard cardiovascular reflex tests. The sensory nerve function was studied with a Neurometer (Neurotron Inc. Baltimore, Md), using transcutaneous electrical stimulation. The digestive complaints were characterized by calculation of symptom scores. **Results:** A positive correlation was observed between the half-time of gastric emptying (HTE) and the AN score ($r=0.59$, $p<0.01$). In accordance with this finding the correlation was negative between the heart rate response to breathing and HTE ($r=-0.45$, $p<0.05$) and between the Valsalva ratio and HTE ($r=-0.46$, $p<0.05$). A positive association was found between orthostatic hypotension and HTE ($r=0.56$, $p<0.01$). The gastric hypomotility was not correlated with the severity of digestive complaints, while a positive correlation was proven between the AN score and the overall digestive symptom score ($r=0.44$, $p<0.05$). Separate analysis of the different complaints revealed a positive correlation between abdominal pain and the AN score ($r=0.42$, $p<0.05$) and between the symptoms of dysmotility and the AN score ($r=0.50$, $p<0.05$). The peripheral sensory nerve dysfunction was not correlated with HTE or the degree of complaints. **Conclusions:** Autonomic neuropathy seems to be the major determinant of gastric hypomotility in type-1 DM. The severity of the digestive symptoms is more frequently associated with the progression of autonomic neuropathy than with the degree of gastric hypomotility or the peripheral sensory nerve dysfunction. These findings may suggest that autonomic neuropathy is primarily responsible for both gastrointestinal hypomotility and the genesis of digestive complaints in DM.

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Aortic distensibility is reduced in diabetic patients with autonomic neuropathy

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Background and Aims: Recent studies have shown an association between cardiovascular risk factors and aortic distensibility (AD) in non-diabetic persons. A few studies have been conducted in persons with diabetes (DP) and, in addition, there is no any information concerning the relationship between diabetic autonomic neuropathy (DAN) and AD in type 2 diabetes.

Materials and methods: Sixty-two subjects with type 2 diabetes as well as thirty-four healthy individuals (C), mean age (SD): 58.3 (9.2) and 54.3 (11.6) years; males n(%): 34 (58.4) and 15 (44.1) respectively; mean duration (SD) of diabetes: 9.1 (7.1) years, without evidence of macrovascular disease, were studied. AD was assessed with high-resolution ultrasonography, 3cm above the aortic valve. Short-term power spectral analysis of the heart rate variability (PSAHRV) and the battery of the classical Ewing tests were used to evaluate DAN.

Results: AD was significantly lower in DP than in C [1.87 (0.44) vs 2.54 (0.29) 10^{-6} dyn $^{-1}$. cm 2 respectively, $P<0.0001$]. In addition, AD was significantly lower in DP with DAN compared to DP without DAN [1.66 (0.54) vs 1.96 (0.54) 10^{-6} dyn $^{-1}$. cm 2 respectively, $P=0.02$]. Multivariate analysis after adjustment for a number of confounding factors such as age, sex, plasma lipids, blood pressure and smoking, showed that AD was related significantly and independently with duration of diabetes [$B=-0.04$, $SE(B)=0.008$, $P<0.0001$] and the total power of the PSAHRV [$B=-0.27$, $SE(B)=0.13$, $P=0.05$].

Conclusions: Patients with type 2 diabetes and especially those with autonomic neuropathy have reduced aortic distensibility. The lack of association between the elastic properties of the aorta and the classical risk factors for atherosclerosis probably indicates that diabetes per se is more important in the initiation and progression of the atherosclerotic process.

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Oxidative Stress is Associated with Abnormal Myocardial Blood Flow Regulation in Subjects with Type 1 Diabetes and Microangiopathy
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Background and Aims: Cardiovascular autonomic neuropathy (CAN) has disabling clinical manifestations and may facilitate malignant arrhythmias by altering electrical stability and/or impairing myocardial blood flow. The etiology of CAN is unknown, but oxidative stress has recently emerged as a contributing factor. The aim of this study was to characterize the relationships of left ventricular (LV) sympathetic dysinnervation and microangiopathy (MA) to altered myocardial vascular responsiveness and oxidative stress in diabetic subjects ± CAN.

Materials and Methods: Quantitative dynamic myocardial blood flow assessment using positron emission tomography (PET) and [^{13}N]-NH $_3$ at rest and during sympathetic stimulation (cold pressor test) was performed in type 1 diabetic subjects with CAN and MA (retinopathy and nephropathy) (n=6), diabetic subjects with MA, but no CAN (n=6), healthy diabetic control (DC) subjects (n=7) and age and sex matched healthy nondiabetic subjects (ND) (n=6). CAN was assessed using a battery of standardized autonomic function tests and PET using [^{11}C]-hydroxyephedrine. Urinary F $_2$ -isoprostanes (a measure of lipid peroxidation) were measured as an index of oxidative stress.

Results: In response to sympathetic stimulation global coronary flow reserve (CFR) increased by 60% and 28% (p<0.05) respectively in ND and DC subjects. In contrast, global CFR decreased by 14% (p<0.05) and 3% in the MA and CAN subjects, respectively. In the CAN subjects, paradoxical vasoconstriction was limited to the proximal innervated myocardial segments. Urinary F $_2$ -isoprostanes levels were markedly elevated in diabetic subjects with MA (51 ± 2 pg/mMol creatinine) as compared with ND subjects (26 ± 2 pg/mMol creatinine (p<0.05)).

Conclusions: In conclusion, diabetic subjects with MA demonstrate increased oxidative stress and paradoxical myocardial vasoconstriction on sympathetic activation. Cardiac dysinnervation, oxidative stress and endothelial dysfunction may predispose to life-threatening myocardial electrical and chemical instability complicating diabetes.

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LEFT VENTRICULAR FUNCTION ASSESSMENT WITH RADIONUCLIDE VENTRICULOGRAPHY AT REST IN RELATION TO AUTONOMIC NEUROPATHY IN TYPE 1 DIABETES MELLITUS.

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Background: A controversy exists about the relation between diabetic autonomic neuropathy (DAN) and left ventricular (LV) function (systolic and diastolic). **Patients – methods:** To investigate this relation, 41 patients with type 1 diabetes mellitus (DM) were studied. We tested 22 women and 19 men with mean age 36.2 ± 12.8 (16 – 66) years and mean duration of DM 18.4 ± 7.8 (6 – 45). The presence of DAN was established if 2 or more of the following 4 standard cardiovascular reflex tests were abnormal. The R-R variation during deep breathing (assessed by expiration/inspiration ratio, mean circular resultant and standard deviation), Valsalva maneuver, 30:15 ratio and blood pressure response to standing were used. LV function was investigated with radionuclide ventriculography (RVN) at rest. Ejection fraction (EF) was used to assess LV systolic function, while peak filling rate (PFR), first third filling fraction (1/3FF), and atrial contribution to ventricular filling (A/V) were used to investigate LV diastolic function. All patients were free from coronary artery disease. Twenty three subjects of similar age were used as normal controls (NC). **Results:** Twenty five patients had not DAN (GROUP A) and the rest 16 had definitive DAN (GROUP B). Heart rate (HR) for all patients with type 1 DM was higher than HR of controls, thus values of diastolic indices were corrected for HR.

	GROUP A	GROUP B	NC	p(A-NC)	p(B-NC)
HR	74.2 ± 10.7	82.4 ± 12.7	65.2 ± 8.6	0.006	0.0001
EF	66.1 ± 6.0	69.9 ± 10.4	64.4 ± 4.8	0.124	0.02
PFR	4.6 ± 1.0	3.8 ± 1.3	4.6 ± 1.3	0.665	0.024
1/3FF	56.2 ± 16.3	37.7 ± 11.0	48.5 ± 12.6	0.142	0.01
A/V	13.4 ± 7.5	21.1 ± 12.7	14.4 ± 11.9	0.841	0.079

Conclusions: It is concluded that LV systolic function is not negatively affected by DM, regardless of DAN presence. LV diastolic function is significantly impaired in patients with type-1 DM and DAN. LV diastolic function is normal in patients with type-1 DM without DAN.

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THE EFFECTS OF LIPOIC ACID ON ANTIOXIDANT STATUS AND PLATELET AGGREGATION IN TYPE 2 DIABETIC PATIENTS WITH CARDIOVASCULAR AUTONOMIC NEUROPATHY

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Background and Aims: Cardiovascular autonomic neuropathy (CAN) affects » 30-40 % of the patients with Type 2 diabetes mellitus. The present study had examined the effect of a-lipoic acid (LA), the lipophilic free radicals "cleaner" on the antioxidant status, platelet aggregation, prostacyclin I $_2$ -thromboxane A $_2$ system in patients with Type 2 diabetes mellitus and CAN. **Materials and Methods:** 49 patients (54±7 years, 27m/22f) were allocated in two treatment groups. All patients were randomized to receive either a daily i/venous (2 weeks) and then per os dose of 600 mg α-LA (n=32) or placebo (n=17) during 2 months. Parameters of platelet function, other biochemical and instrumental investigations were observed at baseline state and at the end of 1 and 2 months period. CAN assessed by reduced heart rate variability (HRV), Ewing's battery tests and QTc interval parameters disturbances. Platelets were exposed to different agonists (1 U/ml of thrombin; 0.1; 0.5 and 5 mM of ADP). ADP-induced platelet aggregation was measured by automatic system. **Results:** Analysis of aggregatory curves shows that platelets in patients with CAN began to aggregate earlier and the speed (0.70 ± 0.01 U/min, p<0.001) and the stage of aggregation (23.60 ± 1.09 MU/min, p<0.01) increases. Obtained results could witness about increase in platelet sensitivity towards thrombin and ADP in Type 2 diabetic patients with CAN. There was an increasing of 12SII-thromboxane B $_2$ (TXB $_2$) level (198.92 ± 11.23 pg/ml, p<0.001), decreasing of 12SII-6-ketoprostaglandin F $_{1\alpha}$ (6-ketoPGF $_{1\alpha}$), (95.9 ± 9.11 , p<0.01) in plasma blood, activity of superoxide dismutase (SOD, 9.14 ± 1.25 IU/ml Rbc's, p<0.001), glutathione peroxidase (GPO, 287.40 ± 21.89 GSH/min 1 g Hb) and catalase in platelets and RBC's, content of reduced glutathione (GSH, 1.66 ± 0.03 mM/1g Hb, p<0.001) simultaneously. After 2 months a course of treatment with LA a decrease of a degree and speed of an aggregation of thrombocytes was marked. Simultaneously, concentration of 6-ketoPGF $_{1\alpha}$, (p<0.001), and activity of SOD, GPO and GSH concentration were authentically augmented, and the contents of TXB $_2$ was considerably reduced (p<0.01). The above-stated changes were accompanied by positive dynamics of tool and functional samples permitting to troubleshoot a cardiac pathology, and, especially, CAN in patients with Type 2 diabetes mellitus. **Conclusions:** a-lipoic acid improves an antioxidant status and platelet aggregation parameters of Type 2 diabetic patients with CAN.

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Postural catecholamine response in Type I diabetes mellitus with chronic renal failure

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Background and Aims: Orthostatic hypotension as a sign of autonomic neuropathy is frequently present in advanced diabetic nephropathy. However, information on catecholamine(CA) kinetics during postural change in diabetic patients with chronic renal failure is scarce. The aim of the study was the assessment of norepinephrine(NE) and epinephrine(E) responses to standing up in Type I diabetic patients(DM) with chronic renal failure or end-stage renal disease.

Materials and Methods: We have examined 14 patients (M/F: 8/6, mean±SD age 44.1 ± 8.8 years, DM duration 22 ± 6 years) from the pancreas and kidney transplant waiting list and 10 healthy sex- and age-matched controls(C). After 30 min in the supine position, systolic(s) and diastolic(d) BP and venous plasmatic levels of NE and E were measured (min -1, 0, 1, 3, 5 and 10) during standing up. BP was measured by cuff sphygmomanometry, CA levels were determined by HPLC (Shimadzu LC-6A pump, Kyoto, Japan) with fluorimetric detection (Shimadzu RF-551 fluorescence monitor) after condensation with diphenylethylenediamine. Data were analysed with the use of ANOVA; Wilcoxon paired test was used for intra-group comparisons, when appropriate.

Results: Apart from sBP (DM vs C: 144 ± 24 vs 112 ± 13 mmHg, p<0.01), no other parameters differed between the groups at rest. BP fell significantly in the DM group after standing up (min 5 - 0: sBP -30 ± 29 mmHg, p<0.001, dBP -9 ± 13 mmHg, p<0.05), while such a response did not occur in the C subjects (min 5 - 0: sBP $+1 \pm 7$ mmHg, DM vs C p<0.01, dBP $+6 \pm 5$ mmHg, DM vs C p<0.001). Blood NE levels increased similarly in both groups (min 5 - 0: DM $+0.85 \pm 0.85$ nmol/l, C $+0.97 \pm 0.85$ nmol/l, DM vs C p>0.05). In both groups, no postural changes of E levels were found. A doubling of NE levels (min 5 vs 0: 2.28 vs 1.13 nmol/l) occurred in 3 DM patients who were unable to remain standing due to severe symptomatic hypotension.

Conclusions: Despite a significant decrease in BP, no changes of catecholamine kinetics during standing up were found in Type I DM patients with chronic renal failure. Other factors may be responsible for orthostatic hypotension in these subjects. Supported by IGA NB/6394-3 and ND/5295-3 grants of the Czech Ministry of Health

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AUTONOMIC NEUROPATHY AND QT-INTERVAL PROLONGATION IN NONALCOHOLIC DIABETICS, NONDIABETIC ALCOHOLICS AND IN ALCOHOLIC DIABETIC PATIENTS

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Background and Aims: Sudden death is not rare in patients with cardiovascular autonomic neuropathy (CAN) and corrected QT-interval (QTcI) prolongation is thought to be important in this respect. **Materials and Methods:** Evaluating the relationship between CAN and QTcI, heart rate responses to deep breathing, standing and Valsalva manoeuvre just as blood pressure responses to standing and sustained handgrip were assessed. QTcI was determined with Bazett's formula. **Results:** 82 controls had normal results in all five tests and a mean QTcI of 397 (SD 21) ms. 70/94 nonalcoholic patients with Type II diabetes mellitus, 14/36 nondiabetic alcoholics and 21/22 alcoholics with Type II diabetes mellitus had CAN. Significant linear regression was found between severity of CAN - based on the number of abnormal reflex tests on patient - and QTcI prolongation ($p < 0.001$ in all three groups). Abnormal QTcI (> 440 msec) was seen significantly more often in patients with CAN compared to those without CAN ($p < 0.001$ in nonalcoholic diabetics and nondiabetic alcoholics, $p < 0.05$ in alcoholic diabetic subjects). Analysing the connection between QTcI and the five reflex tests separately, significant negative correlations indicate that beside the previously established role of sympathetic imbalance even parasympathetic damage contributes to the development of QTcI-lengthening. **Conclusions:** Our data provide evidence of a relation between the presence and severity of CAN and degree of QTcI prolongation in all groups examined. Changes in diabetic patients appear to be attributable to CAN, rather than to diabetes per se.

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Withdrawn

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INCREASED INTESTINAL PERMEABILITY AS A CAUSE OF FLUCTUATING BLOOD GLUCOSE LEVELS.

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Aim: In type 1 diabetic patients postprandial glucose excursions have a major impact on metabolic control. Some type 1 diabetics have highly variable glucose measurements at a given hour of the day in different days under same insulin regimens and very similar diet and physical activity. The present study addresses whether type 1 diabetic patients have increased intestinal permeability and intestinal permeability predicts postprandial blood glucose variability. **Material and methods:** 30 type 1 diabetic patients on intensive insulin treatment (14 M, 16 F, age: 26.7 ± 6.86 , diabetes duration: 5.63 ± 4.6 HbA1c: 9.1 ± 2.5 %) together with 15 age and sex matched healthy controls were enrolled in the study. None of the patients and controls had gastrointestinal disease. All medications except for insulin were withdrawn 48 hours before the test procedure. After an overnight fasting all patients and controls received $100\mu\text{Ci}$ (3.7MBq) of Cr-51 EDTA as a radioactive tracer. The percentage of the isotope excreted in a 24 hour urinary specimen was the permeability measure. Instant blood glucose was measured just before the test and patients performed and recorded self monitoring of blood glucose four times a day (fasting and 2nd hour postprandial levels after each meal) during the following week. Autonomic neuropathy was tested with measurement of R-R interval during deep inspiration and Valsalva maneuver. **Results:** When compared to healthy age matched controls type 1 diabetic patients have increased intestinal permeability. (4.53 ± 1.35 vs 2.3 ± 1.0 %, $p < 0.05$). Increased intestinal permeability correlated with instant blood glucose ($r: 0.38$, $p: 0.03$) and patients with autonomic neuropathy had increased intestinal permeability than others (4.08 ± 1.36 vs 5.56 ± 1.03 %, $p: 0.05$). Intestinal permeability did not change with gender, diabetes duration, HbA1c, total daily insulin dose, glomerular filtration rate, presence microalbuminuria, defecation frequency ($p > 0.05$). Cr-51-EDTA excretion rate significantly correlated with the coefficient of variation of postprandial blood glucose measurements ($r: 0.55$, $p: 0.001$). **Conclusion:** Type 1 diabetic patients have increased intestinal permeability when compared to age matched healthy controls. Intestinal permeability correlates with instant blood glucose and is increased in patients with autonomic neuropathy. Increased intestinal permeability significantly predicts the variability in postprandial blood glucose measurements.

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ERECTILE DYSFUNCTION AND DIABETES: A POPULATION-BASED STUDY

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Background and Aims: Most studies of erectile dysfunction (ED) have been carried out in selected clinical populations, using varying definitions of ED. We conducted a multinational population-based survey in Brazil, Italy, Japan, and Malaysia to study the prevalence and correlates of ED among subjects with varying genetic, cultural, and environmental backgrounds. **Materials and Methods:** In each country, a random sample of approximately 600 men aged 40 to 70 were interviewed about medical history, lifestyle habits, and sexual behavior using a standardized questionnaire (Oct. 1997-June 1998). ED was classified by how often the subject had the "ability to attain and maintain an erection satisfactory for sexual intercourse." Men were classified as normal if the response was "always" or "usually" and as having ED if it was "sometimes" or "never." Men who reported ever having received a diagnosis of diabetes or taking medications for diabetes were classified as "diabetic." Men with no diagnosis of cardiovascular disease, prostate disease, diabetes, ulcer, or depression, nor taking hormones, were classified as "healthy." Age-adjusted odds ratio (OR) and 95% confidence intervals (CI) were calculated for the bivariate associations between ED and other factors. **Results:** The prevalence of ED among diabetic men rose from 25% at age 40-44 to 70% at age 65-70. The age-adjusted OR for ED in diabetic patients vs. healthy men was 2.6 (95% CI=1.9-3.6). Prevalence of ED was 16.1% in healthy men, 31.7% in 104 men with diabetes only (OR=2.4, 95% CI=1.6-3.7), 40% in 30 men with diabetes and heart disease (OR=3.5, 95% CI=1.7-7.3), 46.5% in 71 men with diabetes and hypertension (OR=4.5, 95% CI=2.8-7.4), and 55.6% in 10 men with diabetes and prostate disease (OR=6.5, 95% CI=2.5-16.7). The age-adjusted OR for ED by duration of diabetes (≥ 10 years vs. < 10 years) was 2.0 (95% CI=1.1-3.7). Diabetic men reporting current smoking and lower than average physical activity had a 4-fold increase in the prevalence of ED (OR=3.9, 95% CI=1.4-10.9).

Conclusions: The prevalence of ED in diabetic men is substantially higher than in healthy men the same age. Increasing age, duration of diabetes, presence of concomitant diseases, lack of physical activity, and smoking are significantly associated with this increased prevalence. Funding provided by Pfizer Inc.

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STRATIFYING TYPE 2 DIABETIC PATIENTS AT RISK OF ERECTILE DYSFUNCTION.

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Background and Aims: In the framework of a nation-wide outcomes research program in type 2 diabetes, we estimated the prevalence of self-reported erectile dysfunction (ED) and identified subgroups of patients for whom the interaction among the different factors determined a substantial increase in the risk of ED.

Materials and Methods: The study involved 1460 patients enrolled by 114 Diabetes Outpatients Clinics and 112 General Practitioners. Patients were asked to fill in a questionnaire investigating their ability to achieve and maintain an erection. Clinical information was collected by the participating physicians. CES-D scale was also used to detect depressive symptoms. The main results were obtained by using a tree-growing technique (RECURSIVE Partitioning and AMalgamation - RECPAM), that permits to identify homogeneous and distinct subgroups expressing a different ED risk. **Results:** Overall, 34% of patients referred erectile problems. The RECPAM algorithm led to the identification of seven ED risk classes. The most important variable in discriminating ED risk was represented by diabetes treatment: patients on diet alone showed the lowest probability of ED (prevalence of 19%) and represented the reference category. The subgroup of patients treated with insulin and affected by neuropathy showed the highest risk, with a prevalence of 65% (OR=7.2; 95% CI 3.9-13.2). Among subjects managed with oral agents, levels of risk differed in relation to the presence of depressive symptoms, smoking and cardiovascular (CV) disease; ED prevalence was $> 40\%$ in patients with depressive symptoms (OR=2.7; 95% CI 1.8-3.9) and in current/past smokers with CV disease (OR=2.4; 95% CI 1.5-3.8) while it did not differ from the reference category for non-smokers with no depressive symptoms (OR=1.1; 95% CI 0.7-1.9). Patients' age, tested as continuous global variable, was correlated with ED risk (OR=1.05; 95% CI 1.04-1.07). A final logistic regression also retained the presence of retinopathy (OR=1.6; 95% CI 1.2-2.2) in the final model.

Conclusions: Our data underline the interplay between clinical and psychological factors in determining the risk of ED and can be of help in identifying those patients for whom a much greater attention is needed in the detection of erectile problems.

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Sildenafil in the treatment of erectile dysfunction in an outpatient population of men with type 1 and type 2 diabetes. Demand for treatment, eligibility and results of a 12 week self reported flexible dose escalation study.

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Background and Aims: Erectile dysfunction (ED) is increased in both type 1 and type 2 diabetes, especially after age 40. Sildenafil (Viagra) is an effective oral agent for the treatment of ED irrespective of etiology, but its use and efficacy in ED in diabetes may be limited by late onset complications. The aim of this study was to describe the demand for treatment, eligibility of patients and results of a 12 week self-reported, flexible dose escalation study in an outpatient population.

Materials and Methods: Patient journals of an outpatient population of 187 men over the age of 40 with either type 1 or type 2 diabetes complaining of erectile dysfunction, were evaluated by a diabetologist, followed by written invitation of 118 patients and subsequent screening of 55 patients for treatment with Sildenafil in a 12 week open-label, flexible dose escalation study. Efficacy was assessed using the International Index of Erectile Function, a patient log and a general satisfaction questionnaire.

Results: 69 patients (36.9%) were excluded by journal evaluation, primary exclusion criteria being cardiovascular disease and treatment with nitrates. 49 (41.5%) of the 118 remaining patients approached for the study declined to participate, listing primarily age (44.9%) and concomitant disease (28.6%) as reasons to decline. 33 patients, ages 45-75 years (mean 58.1 \pm 7.2), completed the 12 week intervention. 12 patients (36.4%) reported side effects, but only 1 had to discontinue treatment. 12 patients (36.4%) had satisfactory treatment effect every time they used Viagra after the initial dose adjustment, 9 patients (27.2%) had varied effect, and the remaining 12 patients (36.4%) had no treatment effect at all. The following scores were significantly improved during treatment with Sildenafil ($p < 0.0001$): erectile function, intercourse satisfaction and overall satisfaction.

Conclusions: Sildenafil significantly improved sexual function in men with type 1 or type 2 diabetes and erectile dysfunction, as measured by a validated instrument, that addresses the relevant domains of male sexual function. As expected in a population of men over the age of 40 with diabetes, a large number of patients had to be excluded beforehand or at screening, primarily due to late onset complications, and a considerable group of patients had no treatment effect. As such, Sildenafil is 'no cure all', and patient and partner counseling, including alternative treatment possibilities, is still much needed in the outpatient setting.

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What determines response or non-response to Viagra in diabetic erectile dysfunction? - is it vascular, neuropathic, hormonal or psychogenic factors, or is it severity?

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Background and Aims: Sildenafil (Viagra), a potent inhibitor of phosphodiesterase type 5, has proved an effective oral agent for some diabetic men with erectile dysfunction, but others do not respond. We aimed to use extensive assessments to identify whether any particular aetiological factors were associated with response or non-response to Viagra.

Materials and Methods: We undertook a randomised, double blind crossover study of Viagra (100mg) versus placebo in 31 married men with diabetes complaining of impotence; median age 54 (range 35-73) years, median duration of diabetes 9 (range 2-32) years, median duration of impotence 4 (range 1-10) years. Prior to treatment patients underwent an extensive assessment for diabetic autonomic neuropathy (acetylcholine sweat spot test, pupil test (PD%), battery of five cardiovascular tests), for evidence of a vascular component (erectile response to 60 mg papaverine and 20 μ g alprostadil on separate occasions), for psychogenic factors (detailed assessment of patient, and where possible his wife, by a specialist in psychosexual medicine), for severity of impotence (four grades) and for hormonal factors (testosterone, prolactin).

Results: The results suggested the following aetiological factors: diabetic autonomic neuropathy in 16/31 (52%); probable vascular component in 9/31 (29%), possible vascular component in a further 19/31 (61%); psychogenic factors in 12/29 (41%); depression in 3/31 (10%); testosterone below 11 nmol/l in 12/26 (46%). There were 13 responders to Viagra, 5 partial responders, 11 non-responders and 2 dropouts. Diabetic autonomic neuropathy, evidence of a vascular component, low testosterone and psychogenic factors were equally distributed amongst the responders and non responders, as were duration and severity of erectile dysfunction. All three patients with clinical depression were non responders.

Conclusions: Apart from noting that all 3 patients with clinical depression were Viagra non responders, in our study using extensive investigations, none of the classic factors contributing to diabetic impotence, nor duration or severity of the impotence, seemed to identify response or non-response to Viagra.

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THE EFFECT OF AS-NEEDED IC351 TREATMENT OF ERECTILE DYSFUNCTION IN MEN WITH DIABETES

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BACKGROUND AND AIMS: IC351 is a potent and selective inhibitor of phosphodiesterase type 5. The purpose of this multicenter, double-blind, placebo-controlled study was to assess the efficacy and safety of IC351 taken as needed by diabetic men with mild-to-severe erectile dysfunction (ED). **MATERIALS AND METHODS:** Two hundred sixteen men (mean age: 56 years) with mild-to-severe ED and diabetes (type 1 or 2) were enrolled. Baseline (BL) International Index of Erectile Function (IIEF) scores and Sexual Encounter Profile (SEP) diary information were collected during a 4-week treatment-free run-in period. Patients were randomized to receive placebo (PBO) or IC351 10 or 20 mg for 12 weeks. Endpoints included the percentage of "yes" responses to a global assessment question (GAQ), and the change from BL in IIEF Erectile Function domain scores and SEP diary data. **RESULTS:** IC351 10 and 20 mg were superior to placebo in the percentage of "yes" responses to GAQ (IC351 10 mg [56%] and 20 mg [64%], versus PBO [25%]; $P < 0.001$). Both doses of IC351 significantly improved IIEF Erectile Function domain scores when compared with PBO (change from BL: IC351 10 mg [6.4] and 20 mg [7.3], versus PBO [0.1]; $P < 0.001$). In addition, IC351 10 and 20 mg improved erectile function (IIEF Erectile Function domain) and successful intercourse attempts (SEP Questions 2 and 3) in a greater percentage of diabetic patients than PBO. IC351 10 and 20 mg were generally well tolerated. There were no treatment-related changes in lab values, ECG, or vital signs. Most adverse events were mild to moderate in intensity. The most common treatment-related adverse events occurring in more than 5% of IC351-treated patients were dyspepsia and headache, which were mild to moderate in intensity. **CONCLUSION:** In this study, IC351 10 and 20 mg demonstrated superiority to PBO when administered as needed for the treatment of mild-to-severe ED in diabetic men and was well tolerated.

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 α -LIPOIC ACID IN TREATMENT OF DIABETIC ERECTILE DYSFUNCTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Background and Aims: Erectile dysfunction (ED) is a common problem amongst men with Type 2 diabetes mellitus. The present study has examined the effect of α -lipoic acid (LA, the lipophilic free radicals "cleaner") on the warm temperature discrimination threshold (TDT) in feet, vibration perception threshold on the great toe (VPT), ankle brachial pressure index (ABPI), heart rate variability (HRV), QTc parameters and standard cardiovascular autonomic tests in Type 2 diabetes mellitus with ED. **Materials and Methods:** 78 patients (56 \pm 7 years) were allocated in two treatment groups. All patients were randomized to receive either a daily intra venous (2 weeks) and then per os dose of 600 mg LA (n=47) or placebo (n=31) during 2 months. Parameters were observed at baseline state and at the end of 2 month period. **Results:** All patients with ED had reduced HRV, abnormal cardiovascular autonomic tests (Ewing's score 10.7, total score=1.0); QTc prolongation, higher the mean TDT (8.70 c, $p < 0.001$), abnormal VPT and normal parameters of the ABPI. After 2 months of treatment there was a VPT parameters, root mean square of successive difference (RMSSD, $r=0.61$, $p < 0.05$) and low frequency band, (LF, $r=0.83$, $p < 0.001$) parameters were increased, TDT (5.20 c, $p < 0.001$) and QTc parameters ($r=0.90$, $p < 0.001$) were decreased significantly in patients from 1st group. Also, we observed more significant improvement of cardiovascular autonomic tests. Efficiency of LA was also valued in agreement with questions, shown in International Index of Erectile Function. After 2 months treatment course with LA, 79.8 % of the patients have answered positively to the question: "Did treatment improve your erection?". The general index in the 1-st group, including the answer to a question about achieving and maintaining of an erection, has appeared considerably higher ($p < 0.01$). **Conclusions:** ED is strongly associated with CAN. LA may be used for treatment of erectile dysfunction in patients with Type 2 diabetes mellitus.

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Which is the more effective intracorporeal agent for diabetic erectile dysfunction - 60mg papaverine or 20µg prostaglandin E1 (alprostadil)?

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Background and Aims: Despite the emergence of sildenafil (Viagra) as an orally active agent for diabetic erectile dysfunction, there remain many patients who do not respond to this agent or for whom there are contraindications such as nitrate treatment for ischaemic heart disease. Self-injected intracorporeal vasoactive agents remain an important part of the therapeutic armamentarium. The direct acting smooth muscle relaxant papaverine was the original agent used. It is cheap and has had extensive clinical use despite not having marketing authorisation. Prostaglandin E1 (alprostadil) is a newer vasoactive agent; it has marketing authorisation, but it is also more expensive than papaverine. It is therefore important to know if there is a therapeutic difference between the two agents. We aimed to compare the efficacy of alprostadil (Caverject) with papaverine sulphate in diabetic men with impotence.

Materials and Methods: 30 married men with diabetes complaining of impotence were recruited to the study. The mean age was 54 years (range 35-73), duration of diabetes 9 yrs (range 2-32), and duration of impotence 4 yrs (range 1-10). All patients had the intracorporeal injection of alprostadil and papaverine on separate days in random order. The response to the medication was graded from grade 0 (no reaction) to grade 3 (full erection). For papaverine the dose used was 60mg (30mg followed by a further 30mg 15 minutes later) and for alprostadil the dose was 20µg (10µg followed by a further 10µg 15 minutes later).

Results: Only 3/30 (10%) patients had a full erection (grade 3) response to papaverine whereas 13/30 (43%) had a full erection following alprostadil. 2/30 (7%) had no response (grade 0) to papaverine whereas there were no patients with a grade 0 response to alprostadil. The other grades of response, papaverine versus alprostadil, were grade 2, 17/30 (60%) versus 12/30 (40%); grade 1, 7/30 (23%) versus 12/30 (40%). Thus patients had a significantly greater erectile response with alprostadil than with papaverine. The median difference in scores was 0.5 (95% CI 0 to 1.0), $P=0.007$.

Conclusions: The erectile response to 20µg of alprostadil in diabetic men with impotence is significantly greater than that to 60mg papaverine. Though alprostadil is more expensive than papaverine, it does have marketing authorisation and as it also appears from our study to be more effective, it is probably the preferred agent for clinical use.

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IC351 ENHANCES NITRIC OXIDE-MEDIATED RELAXATION OF HUMAN ARTERIAL AND TRABECULAR PENILE SMOOTH MUSCLE

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BACKGROUND AND AIMS: The nitric oxide/cyclic GMP (NO/cGMP) pathway is central to the neurogenic relaxation of penile smooth muscle needed to achieve and maintain erection. Inhibition of cGMP degradation by inhibiting phosphodiesterase type 5 (PDE5) activity is a target for treatment of erectile dysfunction (ED). This study evaluated the selectivity of the novel PDE5 inhibitor IC351. In addition, its effect on NO-mediated relaxation, and cGMP accumulation in human trabecular penile smooth muscle was assessed. **MATERIALS AND METHODS:** IC351 selectivity for various human PDEs was determined using human recombinant PDEs 1A-C, 2, 3A-B, 4A-D, 5, 7-10, and of PDE6 from human retinas, using *in vitro* PDE assay. Human corpora cavernosa tissues were collected from men with ED during penile prosthesis implantation. Penile arteries from these tissues were mounted on Halpern-Mulvaney myographs. Strips of cavernosal smooth muscle were placed in organ baths. Sodium nitroprusside was used to directly activate the endogenous guanylyl cyclase. Transmural electrical stimulation (0.5-12.0 Hz) was used to evaluate neurogenic relaxation of trabecular smooth muscle. **RESULTS:** IC351 potently and selectively inhibited PDE5 activity. In comparison to IC351's inhibition of PDE5 (IC₅₀ 0.9 nM), a 780-fold higher concentration of the compound is needed to inhibit the retinal enzyme PDE6 (IC₅₀ 730 nM). Still greater concentrations of IC351 are needed to obtain any appreciable inhibition of other human PDEs (PDE1-4, 7-10, >9000-fold). Neurogenic relaxation of trabecular smooth muscle was enhanced by IC351 (30 nM), producing a significant increase of maximum relaxation to nitrergic nerve stimulation (67.4 \pm 10.8% versus 42.4 \pm 8.2% at 6 Hz). IC351 treatment (30 nM) produced a significant potentiation of cGMP accumulation produced by sodium nitroprusside (1 µM) in human cavernosal tissue (cGMP; 0.99 \pm 0.18 versus 0.47 \pm 0.12 pmol/mg protein, n=6). IC351 also potentiated the relaxation of penile smooth muscle induced by acetylcholine.

CONCLUSIONS: In this study, IC351 demonstrated potent and selective inhibition of PDE5. IC351 potentiated responses mediated by exogenous and endogenous sources of NO in human penile smooth muscle by promoting cGMP accumulation. IC351 is presently being evaluated as an oral therapy for ED.

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Neuropathy: Experimental

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A METABOLIC MODEL OF RAT SCIATIC NERVE SORBITOL.

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Background and Aims: Sorbitol is produced by aldose reductase (AR) and consumed by sorbitol dehydrogenase (SDH). Reduction of nerve sorbitol has been used as a key endpoint to assess inhibition of nerve AR in patients with diabetic neuropathy. We aimed to create and evaluate a model of nerve sorbitol, $[S]_{ss}$, to define its dependence on AR inhibition. **Materials and Methods:** At steady-state in a single compartment where coenzyme concentrations are not rate limiting, (1):

$$[S]_{ss} = (K_{ms}k_{AR}[AR][G]_{ss}) / (k_{SDH}[SDH]K_{mG} + k_{SDH}[SDH][G]_{ss} - k_{AR}[AR][G]_{ss})$$

where, respectively, K_{mG} and K_{ms} are Michaelis constants of AR for glucose and of SDH for sorbitol, k_{AR} and k_{SDH} are catalytic rate constants, $[AR]$, $[SDH]$ and $[G]_{ss}$ are tissue concentrations of AR, SDH, and (straight-chain) glucose. $[AR]$ and $[SDH]$ in homogenates of sciatic nerve from normal and streptozocin-diabetic rats were measured on Western blots with antisera raised to human rAR and rSDH. Enzyme kinetic constants of rat rAR and rSDH were determined at 37°C and pH 7.1 by standard methods. Values for $[G]_{ss}$ and $[S]_{ss}$ were taken from literature. **Results:** $[AR]$ and $[SDH]$ in normal and diabetic nerves were similar and were pooled: $[AR]$, $9.1 \pm 2.7 \mu M$ (16); $[SDH]$, $1.5 \pm 0.8 \mu M$ (13). K_m and k_{cat} for straight-chain glucose with AR: $4.9 \mu M$ and 32.7 min^{-1} , respectively. K_m and k_{cat} for sorbitol with SDH: 1.6 mM and 104 min^{-1} , respectively. Calculation of $[S]_{ss}$ from (1) with the assumption that $[AR]/[SDH]$ activity = 24 gave a good fit of published data ($R \sim 0.9$). Of particular note, the relationship between $[S]_{ss}$ and $[AR]$ was nonlinear at $[AR]/[SDH]$ ratios greater than 10. **Conclusions:** These results suggest that a high degree of inhibition of sorbitol, i.e., >95%, corresponds to a similar degree of AR inhibition, but lesser reductions in $[S]_{ss}$, e.g., ~80%, may overestimate the extent of neural AR inhibition.

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EFFECT OF CERIVASTATIN ON NERVE DYSFUNCTION IN DIABETIC RATS.

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Background and aim: We have shown that in diabetic rats, cerivastatin may prevent the increase of capillary filtration of albumin. The aim was to study the effect of cerivastatin on motor and sensory nerve conduction velocities (MCV and SCV) and duration of the sensory nerve potential in rats with STZ-induced diabetes. **Material and methods:** 40 STZ rats aged 82 ± 6 days were randomised in 2 groups, one treated by cerivastatin (group T), the other untreated (group U). The electrophysiological measurements were repeated twice; at mean age of 3 months, before treatment (T0) and after 5 months of treatment (T1). **Results:** At T0, in diabetic animals, MCV and SCV and duration of the sensory nerve potential did not differ significantly from control animals: respectively $41.4 \pm 0.5 \text{ m/s}$ vs $42.2 \pm 0.8 \text{ m/s}$; $54.1 \pm 0.6 \text{ m/s}$ vs $54.2 \pm 1 \text{ m/s}$; and $2.7 \pm 0.03 \text{ s}$ vs $2.5 \pm 0.01 \text{ s}$. At T1, MCV was 45.2 ± 1.4 , 47.2 ± 1.1 and $51.1 \pm 0.8 \text{ m/s}$ respectively in group U, T and in controls, SNV was 60.4 ± 1.4 , 62.1 ± 1.6 and $64.4 \pm 0.7 \text{ m/s}$ respectively; duration of the sensory nerve potential was 2.6 ± 0.07 , 2.4 ± 0.05 and $2.3 \pm 0.08 \text{ s}$ respectively. The three parameters differed significantly between group U and controls ($p < 0.01$) but not between group T and controls. **Conclusion:** This preliminary study suggests a beneficial effect of cerivastatin in the protection against diabetic injury of large fibers. This effect, associated with the microcirculatory effect, plays a role of cerivastatin in endothelial and nerve function protection in diabetes.

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EFFECT OF NICOTINAMIDE AND NICOTINOYL-GABA ON BRAIN GABA-BENZODIAZEPINE COMPLEX IN STREPTOZOTOCIN-INDUCED DIABETES

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Background and Aims: For a better understanding of biochemical mechanisms underlying diabetic encephalopathy the present study was designed to investigate the functional state of brain GABA-benzodiazepine complex in diabetes and elucidate how NAM and nicotinoyl-GABA (picrotoxin, P) may potentially regulate its functioning. **Material and Methods:** All studies were carried out after four weeks of STZ-induced diabetes in rats treated with or without NAM or P (200 mg/kg daily, injected i.p. for 10 days). Brain synaptosomes and synaptic membranes were obtained by stepwise ultracentrifugation in a sucrose gradient. Specific binding of ligands was assessed by radio-ligand assay. **Results:** Studies of $[U-^{14}C]$ GABA uptake by brain synaptosomes have demonstrated its elevation by 60% in STZ-diabetic rats as compared to control. GABA uptake was normalized after P administration and slightly decreased after NAM treatment. To evaluate the functional state of GABA receptors a pharmacologically active GABA analogue muscimol was used. The findings have shown twofold lowering effect of diabetes on $[^3H]$ muscimol specific binding by brain synaptic membranes. P exerted negligible stimulating effect on this parameter, whereas effect of NAM administration was statistically insignificant. Despite the impairment of inhibitory function of GABA-ergic system in diabetes a crucial activation of benzodiazepine receptors was determined as it was shown a threefold increase in binding of $[^3H]$ flunitrazepam by synaptic membranes. Exposure of synaptic membranes to exogenous $10^{-4} M$ GABA (in vitro studies) resulted in a marked elevation of $[^3H]$ flunitrazepam specific binding in control but had no influence in diabetes. These results are in line with assumption that diabetes-induced alterations exists in coupling GABA and benzodiazepine receptors that might be accompanied by changes in conformational state of this membrane-bound complex. Both P and to a less extent NAM treatment resulted in a reduction of a number of benzodiazepine binding sites that led to normalization of $[^3H]$ flunitrazepam specific binding. In contrast with diabetes, exogenous GABA has activated benzodiazepine receptors in membranes obtained from treated rats. **Conclusions:** Our study suggests that NAM and especially P play a role in improving the functioning of brain GABA-benzodiazepine complex impaired in diabetes through specific ligand-mediated mechanism and can be useful in the management of diabetes-associated brain failures.

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CHANGES IN CALCIUM SIGNALLING IN RAT NOCICEPTIVE NEURONS DURING EXPERIMENTALLY-INDUCED DIABETES

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Background and Aims: Alterations in Ca^{2+} signalling responsible for synaptic transmission of nociceptive volleys may be one of the mechanisms leading to neuropathic complications; therefore the possibility of such changes were studied in nociceptive sensory neurons of rats with experimentally-induced diabetes mellitus. **Materials and Methods:** Control and streptozotocin-treated diabetic rats with serum glucose level of 16-25 mM were used. Ca^{2+} transients evoked by high-potassium depolarization were measured fluorometrically in primary nociceptive neurons isolated from dorsal root ganglia and secondary neurons from spinal dorsal horn. **Results:** Ca^{2+} transients induced in these neurons by membrane depolarization did not reveal substantial difference in their peak amplitudes in control and diabetic conditions. However, a definite prolongation of the decay phase of transients was observed in diabetic conditions. The residual $[Ca^{2+}]_i$ elevation measured 60 sec after termination of depolarization reached in primary neurons $17.7 \pm 2.9\%$ of the peak value compared with $6.8 \pm 0.8\%$ in control, and in secondary neurons $14.1 \pm 1.2\%$ compared with $2.8 \pm 0.5\%$ in control. To analyze the mechanisms of these changes, alterations in the Ca^{2+} -accumulating function of endoplasmic reticulum and mitochondria were analyzed. In control primary nociceptive neurons no Ca^{2+} uptake by ryanodine- or InsP₃-sensitive stores could be revealed, and in this case only release of Ca^{2+} from mitochondria could be responsible for prolongation of the calcium signal. Switching-off of mitochondrial Ca^{2+} accumulation by CCCP abolished such prolongation. In secondary nociceptive neurons effective Ca^{2+} uptake by the reticulum was present, but in diabetic conditions substantially decreased: application of caffeine increased $[Ca^{2+}]_i$ by only $41 \pm 9 \text{ nM}$ compared with $268 \pm 29 \text{ nM}$ in control, $P < 0.01$. Thus in this case the failure of both endoplasmic reticulum and mitochondria may participate in the retardation of Ca^{2+} elimination from the cytosol.

Conclusions: Substantial prolongation of calcium transients induced by excitation of nociceptive neurons is a regular sequence of diabetic hyperglycemia and in fact may be the reason for neuropathic potentiation of synaptic transmission of pain signals. The work was funded by grants from Juvenile Diabetes Found. Internat. 1-2000-31 and INTAS-99-01915 and approved by Bogomoletz Inst. Clinic of Animal Care and Use.

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SMALL FIBER NEUROPATHY IN IMPAIRED GLUCOSE TOLERANT GK-RATS

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Background and Aims: Differences between diabetic neuropathy (DN) in the two types of diabetes have been reported, suggesting that factors in addition to hyperglycemia are involved in its pathogenesis.

Materials and Methods: In this study, the functional and structural neuropathy was examined in 18 mo impaired glucose tolerant (IGT) GK-rats and 18mo control.

Results: GK rats showed decreased body weight (454 ± 31 vs 627 ± 80 g; $p < 0.001$), increased blood glucose levels (4.4 ± 1.3 vs 3.2 ± 0.4 mmol/l; $p < 0.02$) and impaired glucose tolerance ($p < 0.001$). IGT rats showed decreased nerve conduction velocity (49.8 ± 5.1 vs 61.8 ± 4.9 m/sec; $p < 0.001$). Sural nerve morphometry showed increased fascicular area ($p < 0.01$), normal fiber number (N.S) and decreased fiber density ($p < 0.001$), indicating endoneurial edema. Myelinated fiber parameters showed decreased axon/myelin ratio ($p < 0.05$), normal mean axonal area (N.S), increased mean fiber area ($p < 0.001$) and myelin area ($p < 0.01$). These data suggest that small myelinated fibers are preferentially affected with preservation of large myelinated fibers. This was confirmed by fiber size distribution histograms which showed a significant relative increase in fibers $> 45 \mu m^2$ in diabetic rats (42.6 ± 2.9 vs 29.8 ± 3.8 ; $p < 0.001$).

Conclusions: These findings differ from those in type 1 or type 2 diabetic neuropathy (STZ-, BB/W-, and BB/Z-rats), which are characterized by fiber loss (STZ- and BB/W-rats) and axonal atrophy affecting all fiber categories and lack of endoneurial edema in the chronic stage of neuropathy. These findings are consistent with those in IGT humans. We conclude that long-term IGT affects selectively small myelinated fibers, rather than all fiber categories.

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Effects of rosuvastatin on small nerve fibre function, ganglion blood flow and endothelium-dependent relaxation in diabetic rats.

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Background and Aims: Impaired nerve perfusion contributes to the aetiology of diabetic neuropathy and elevated blood lipids are a neural and vascular risk factor. In a previous study, we demonstrated beneficial effects of the potent statin, rosuvastatin (AstraZeneca), on large myelinated nerve fibre function in experimental diabetes. The aim was to extend this analysis to small nerve fibre function, ganglionic perfusion and endothelial vasodilator function. **Materials and Methods:** Diabetes was induced by streptozotocin (45 mg/kg) in mature male Sprague-Dawley rats. After 4 weeks, corpus cavernosum and mesenteric vascular beds were removed for in vitro study. In separate groups of rats, studied after 6-8 weeks of diabetes, pain fibre-mediated dysfunction was estimated using a behavioural test for thermal (plantar test) hyperalgesia. In final experiments, under butabarbital anaesthesia, superior cervical ganglion blood flow was estimated by hydrogen-clearance microelectrode polarography. In treated diabetic groups, rosuvastatin (20 mg/kg p.o.) was given preventively or in an intervention study for 2 weeks after 6 weeks of untreated diabetes. **Results:** Adrenergic nerve-mediated contraction of corpus cavernosum was $48.6 \pm 6.6\%$ reduced ($p < 0.001$) by 4 weeks of diabetes. Rosuvastatin treatment attenuated this deficit by $71.2 \pm 12.4\%$ ($p < 0.01$). Nitrogenic nerve-mediated relaxation of phenylephrine-precontracted corpus cavernosum was isolated in the presence of guanethidine and atropine. Diabetes caused $24.9 \pm 6.7\%$ ($p < 0.001$) reduction in maximum relaxation and this was $69.8 \pm 22.6\%$ ($p < 0.02$) prevented by rosuvastatin treatment. Cumulative dose-response data from mesenteric vessels revealed an approximately 54% diabetic deficit ($p < 0.001$) in acetylcholine-stimulated vasorelaxation mediated by endothelium-derived hyperpolarizing factor, which was completely prevented ($p < 0.01$) by rosuvastatin treatment. A diabetic deficit ($52.5 \pm 3.1\%$) in superior cervical ganglion blood flow was $96.7 \pm 11.2\%$ corrected ($p < 0.001$) by 2 weeks rosuvastatin treatment. Diabetic rats exhibited thermal hyperalgesia, evidenced by a $33.7 \pm 3.4\%$ ($p < 0.001$) reduction in foot withdrawal latency to noxious stimulation, which was $86.7 \pm 15.7\%$ ($p < 0.01$) corrected rosuvastatin. **Conclusions:** Diabetes caused dysfunction of small autonomic and pain fibre systems, which was markedly attenuated by rosuvastatin treatment. It is plausible that elevated neural blood flow consequent on improved endothelium-dependent relaxation contributed to these beneficial effects, which could be the subject of further investigation in man.

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MOLECULAR PATHOLOGY OF THE NODE OF RANVIER IN TYPE I DIABETIC NEUROPATHY: A ROLE FOR INSULIN AND C-PEPTIDE DEFICIENCIES?

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Background and aims: C-peptide replacement in type I BB/W-rats prevents the metabolic and chronic structural changes of the node of Ranvier. Nodal integrity depends on specialized molecules including ankyrinG and caspr. AnkyrinG interacts with Na⁺ channels, Na⁺K⁺-ATPase, L1 and NCAM sequestering them to the node. Caspr localizes to the axoglial junctions responsible for the specializations at the node. The insulin receptor (IR) is localized to paranodal membranes, suggesting that insulin signaling is critical to nodal activity. In addition, insulin signaling is potentiated by C peptide. In vitro studies suggest an interaction between caspr and PI3K via a SH3 domain. We hypothesize that these molecules are altered in type I neuropathy and that insulin and/or C peptide deficiencies underlie these changes.

Materials and methods: Sciatic nerves from 2 and 8 mo type I BB/Wor-rats, C-peptide treated and control rats were used for RT-PCR, immunoblotting and immunohistochemistry. Human neuroblastoma cells were used for immunoprecipitation studies. **Results:** Immunoblotting (sciatic nerve) and semi-quantitative RT-PCR (DRG) revealed no differences in ankyrinG and its associated molecules between control; C peptide treated (75ng/kg/d), and untreated diabetic BB/W-rats at 2 and 8 mo. AnkyrinG is post-translationally modified by O-linked N-acetylglucosamine (O-GlcNAc). Immunoprecipitation of ankyrinG in SH-SY5Y cells treated with C peptide (3.3nM), insulin (4.0nM), or both for 2 hr. showed increased O-GlcNAc modification of ankyrinG with insulin only. Cells exposed for 20 hr showed decreased O-GlcNAc modification by insulin alone but was enhanced with C peptide. Therefore, C peptide potentiates the effect of insulin long term (20 hr) but blunts its effects short term (2 hr). Immunoblotting (sciatic nerve) and semi-quantitative RT-PCR (DRG) revealed no difference in the expression level of caspr at 2 mo. At 8 mo caspr protein expression was decreased in diabetics but increased in C peptide treated animals. Immunohistochemical studies showed that caspr is laterally displaced from the paranode in diabetic rats at 2 mo, which is prevented by C peptide. **Conclusions:** These findings suggest that localization and post-translational modifications may be more important than alterations in the expression of these nodal molecules in type I diabetic neuropathy.

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THE EFFECT OF C-PEPTIDE ON CELL PROLIFERATION AND APOPTOSIS IN HUMAN NEUROBLASTOMA CELLS

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Background and Aims: Previous studies have indicated that C-peptide has anti-apoptotic and neuroprotective functions in type I diabetic BB/W rats. In this study we examined the anti-apoptotic and mitogenic effect of C-peptide in human neuroblastoma SH-SY5Y cells and explored possible mechanism.

Materials and Methods: SH-SY5Y cells were exposed to C-peptide alone or in combination with insulin. Cell proliferation was monitored by an electric coulter counter and neurite outgrowth was measured by an image analysis system with image-pro plus 3.0 program. Apoptosis of SH-SY5Y cells were induced by high concentration of glucose and confirmed by DNA fragmentation. Immunoblotting (IB) and immunoprecipitation (IP) of the cell lysate were applied.

Results: The increase in cell number (4 days) with C-peptide alone was: 164 ± 9 ($\pm SD$); 188 ± 6 ; and $172 \pm 10\%$ ($p < 0.001$) for 1.0, 3.3 and 10 nM respectively. The combination of maximal C-peptide and insulin doses significantly ($225 \pm 13\%$; $p < 0.05$) increased cell proliferation over that of C-peptide alone. The average length of neurite outgrowth increased when C-peptide was added together with insulin ($P < 0.001$). Upon addition of C-peptide to high glucose (150 or 300 mM) culture medium, the extent of apoptotic cells was reduced. IB and IP showed increased phosphorylation of insulin receptor (IR) and MAP kinase by C-peptide addition to insulin, indicating C-peptide enhances insulin activity and its downstream signaling. Increased p38 phosphorylation and decreased JNK phosphorylation also was induced by C-peptide which may be involved in the IGF-I receptor (IGF-IR) mediated anti-apoptotic effect.

Conclusion: C-peptide appears to have an insulin-like mitogenic effect on cell proliferation and neurite outgrowth in addition to anti-apoptotic effect in this in vitro system. These effects may be mediated by enhanced activity of the IGF-IR / IR signaling pathways.

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RISK FACTORS ASSOCIATED WITH SYMPTOMATIC PERIPHERAL NEUROPATHY IN A DIABETES POPULATION IN TANZANIA: A CASE-CONTROL STUDY

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Background: Although peripheral neuropathy (PN) remains a significant cause of morbidity in adult diabetes patients in sub-Saharan Africa, associated risk factors for PN in this patient population remain unknown.

Aims: (1) To characterize the epidemiology of PN and (2) document risk factors for PN in an adult population attending the diabetes outpatients clinic at Muhimbili National Hospital, Dar es Salaam, Tanzania.

Material and Methods: During 1/97-12/00 (study period), we conducted a case-control study. Non-selective, consecutive, adult diabetes patients were enrolled after informed consent. Detailed clinical and epidemiological data were recorded for each patient followed by a comprehensive physical examination. A case-patient was defined as any diabetes patient with symptom and/or signs of PN. Control-patients were randomly chosen diabetes patients with no history or clinical evidence of PN.

Results: Two hundred patients met the case-definition and 206 control-patients were identified. Case-and control-patients were similar for region of residence, presence of micro- and macrovascular disease, alcohol use, or diabetes type. However, compared with control-patients, case-patients had significantly higher median duration of diabetes (188 vs 21 months, $p < 0.05$), median age (54 vs 50 years, $p < 0.001$), or lower median body mass index (24 vs 27, $p < 0.001$). On multivariate analysis, factors that were independently associated with PN included African race ($p < 0.001$), male sex ($p < 0.001$), duration of diabetes ($p < 0.001$), tobacco intake ($p < 0.01$), presence of a foot ulcer ($p < 0.05$), or body mass index > 30 . On stratified analysis, the only independent risk factor for foot ulcers was type 1 diabetes ($p < 0.001$).

Conclusions: We have identified independent risk factors for PN and foot ulcers in an adult diabetes population in Tanzania. Patient management algorithms should reflect these factors. Initial preventive efforts should be targeted towards male smokers with type 1 diabetes and relatively low BMI

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Increased Incidence of Diabetic Neuropathy on Smokers: The Sheffield Prospective Diabetes Study

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Background and Aims: A number of macrovascular risk factors have been associated with the development of diabetic neuropathy (DN), however, the relationship of smoking to DN has not been clearly established within the context of a prospective study. The aim of Sheffield Prospective Diabetes study was to identify the early abnormalities of clinical, biochemical, neurophysiological and haemorrhheological functions for the development of complications of type 1 diabetes.

Materials and Methods: 66 newly diagnosed diabetes subjects (mean age 31 \pm 9 (SD) duration (3 years \pm 2) were identified and followed up for 9 years. They had detailed smoking history and neurological assessment (symptoms and sign score, nerve conduction, vibration perception threshold, warm thermal discrimination threshold and cardiac autonomic function test) done at baseline and at follow up. Dyck's criteria were used to define DN. 9 subjects who had high c-peptide and 10 subjects who had neuropathy at baseline were excluded from the analysis.

Results: 40 subjects (12 smokers and 28 non-smokers at baseline) were followed up at 9 years and 7 (17.5%) developed DN. Smokers at baseline were more likely to develop DN than non-smokers (41.6% vs 7.1%; $p < 0.0001$) and this was significant after adjusting for the HbA1c. Cigarette smoking was associated with significant ($p < 0.05$) reduction in sural nerve conduction and rise in vibration perception threshold. Smokers were also likely to develop microalbuminuria ($p < 0.0001$) and there was a trend for the development of retinopathy ($p = 0.07$).

Conclusions: This prospective study which has carefully defined the presence and extent of neuropathy, confirms that smoking is a significant risk factor for the development of DN suggesting a vascular aetiology. Diabetic subjects who smoke should be actively encouraged to stop this to prevent/reduce the likelihood of both macrovascular and microvascular complications.

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Peripheral neuropathy is associated with raised plasma triglyceride levels - Cross-sectional analysis of a diabetic clinic population.

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Background and Aims: The association between neuropathy and increased mortality in diabetes is well known. The causal association is however speculative and mainly linked to autonomic abnormalities. The main aim of this study was to try and identify possible metabolic abnormalities related to this complication

Materials and Methods: The analysis was performed on all 1908 patients attending our clinics between 31.01.00 and 01.02.01. The mean age of patients was 60.2 (SD \pm 15.7) years and the mean duration of diabetes was 12.7 (SD \pm 12.7) years. Peripheral neuropathy was defined as a Toe VPT (Horwell Neurothesiometer) > 25 volts. The following plasma metabolic measurements were studied using ANOVA and student's t test: Fasting glucose, glycated haemoglobin (HbA1c), total cholesterol, HDL cholesterol, LDL cholesterol, fasting triglycerides and creatinine.

Results: 480 patients (25%) had a VPT > 25 volts. These patients with neuropathy had higher triglyceride levels (2.16 \pm 1.4 mmol/L vs. 1.90 \pm 1.16 mmol/L, $p < 0.0001$) and lower HDL cholesterol levels (1.18 \pm 0.38 mmol/L vs. 1.28 \pm 0.57 mmol/L, $p = 0.0002$) than those with a VPT < 25 volts. Creatinine was also positively associated with peripheral neuropathy (116 \pm 67.5 vs 98.9 \pm 29.8, $p < 0.0001$). Fasting glucose, HbA1c, Total cholesterol and LDL cholesterol showed no significant correlation with this complication (p values 0.27, 0.89, 0.62 and 0.63 respectively)

Conclusions: This interesting observation may partly explain a causal association between neuropathy and increased cardiovascular risk related to raised triglyceride levels in diabetes. Hypertriglyceridaemia has been implicated as a cause of a distinct type of non-diabetic neuropathy and may also be contributory to the development of neuropathy in diabetic patients.

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CANDIDATE GENE POLYMORPHIC MARKERS AND ASSOCIATION WITH DIABETIC NEUROPATHY IN RUSSIAN IDDM PATIENTS

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Background and Aims: In this research an association of diabetic neuropathy (DN) with polymorphic markers located within six candidate genes: catalase gene (*CAT*) - *C1167T* polymorphism, mitochondrial superoxide dismutase gene (*SOD2*) - *Ala(-9)Val* polymorphism, extracellular superoxide dismutase gene (*SOD3*) - *Arg213Gly* polymorphism, glutathione peroxidase gene (*GPX1*) - *Pro197Leu* polymorphism, neuronal NO synthase gene (*NOS1*) - polymorphic STR located at the exon 29 and aldose reductase gene (*ALR2*) - polymorphic STR located at the 5'-region, have been studied. **Materials and Methods:** To overcome probable predominance and masking effects of metabolic risk factors on DN phenotype expression, we have formed two groups of patients with type 1 diabetes: DN+ group ($n=42$) with the overt diabetic neuropathy and DN- group ($n=87$) in which all patients had no clinical neuropathy. To identify alleles of polymorphic markers we used PCR technique in combination with restriction endonuclease cleavage and gel electrophoresis. **Results:** We have not found statistically reliable differences in allele and genotype frequencies between DN+ and DN- groups in case of polymorphic markers of *SOD2*, *GPX1* and *ALR2* genes. In case of *NOS1* gene the allele 16 and genotype 16/16 have been risk factors ($OR = 1.45$ and 2.34 , respectively). In case of *SOD3* gene *Arg/Arg* genotype was significantly higher (5.05 times) in DN+ group in comparison with DN- group ($OR = 5.09$). All these differences were statistically reliable ($p < 0.05$). The study of allele distributions of *C1167T* marker of *CAT* gene have shown that the prevalence of the C allele was higher (1.3 times) and T allele was lower (1.8 times) in the DN+ group in comparison with DN- group and these differences were statistically reliable ($p < 0.05$). **Conclusions:** Our data allow us to suppose that polymorphic markers of *NOS1*, *SOD3* and *CAT* genes can be associated with development of diabetic neuropathy among patients with type 1 diabetes in a Moscow population.

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ASSESSMENT OF CEREBRAL METABOLISM IN VIVO IN PATIENTS WITH TYPE 1 DIABETES MELLITUS

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Background and Aims: The brain's metabolism and functions could be affected in patients with diabetes mellitus. However, this complication of disease is commonly overlooked and there is no data regarding the cerebral metabolism assessed in vivo in diabetic patients. Therefore, the purpose of the study was to investigate cerebral metabolism in patients with long-term type 1 diabetes mellitus by in vivo magnetic resonance spectroscopy (MRS).

Materials and Methods: We studied 12 diabetic patients (age: 29.3±8.0 years, diabetes duration: 12.6±3.50 years; HbA1c: 8.4±1.35%, mean±SD) and 19 control subjects (age: 32.6±5.2 years). There were no clinical signs of overt cerebral disorders in subjects studied. The content of cerebral metabolites (N-acetylaspartate (NAA), total creatine (Cr) and choline-containing compounds (Cho) were measured in the gray matter of brain occipital region by IH MRS using Magnetom Vision Plus System. Selection of regions studied was made by T1 and T2-weighted images in the axial plan. Statistical analysis was performed by Student's paired test.

Results: We found significant decrease of NAA peak levels in brain in diabetic patients - 39.6±19.9 vs. 52.6±11.6 and 37.2±16.5 vs. 50.9±13.1 in diabetic and control subjects in left and right hemisphere, respectively, $p<0.05$. Moreover, it was decrease of cerebral Cr in left occipital region in patients with diabetes (17.3±9.2 vs. 26.3±6.6, $p<0.05$) while the changes of Cr in right (20.7±15.1 vs. 27.6±10.7, $p>0.05$) and Cho in both hemispheres were not statistically significant. Furthermore, the traces of glucose and lactate in the brain were detected more frequently in diabetic patients compared to controls.

Conclusions: The revealed changes of cerebral metabolites content could reflect the disturbances of brain's metabolism in patients with long-term diabetes and their clinical significance requires further investigation.

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LAMININ LEVELS ARE REDUCED IN PROAPOPTOTIC SERA OF PATIENTS WITH DIABETIC NEUROPATHY.

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Background and Aims: Laminin (Lm) exerts a neuroprotective, antiapoptotic effect on neuronal cells (NIE-115 neuroblastoma) in culture exposed to toxic sera from patients with diabetic neuropathy (DN). We hypothesized that Lm levels would be low in patients with DN.

Materials and Methods: In order to test this hypothesis, we studied serum Lm levels by ELISA in 27 neuropathic patients with type 2 diabetes mellitus, 20 patients with autoimmune neuropathies (AIN) and 15 healthy controls.

Results: In sera of patients with DN, Lm was significantly lower than in patients with AIN (537.99±35.4 vs. 674.10±41.1 ng/ml, $p=0.045$), but not significantly lower than controls (634.10±23.9 ng/ml). There were no significant differences between DN patients and AIN patients regarding age, neuropathy duration, blood pressure, and the severity of neuropathy. HbA1c and BMI were significantly higher in type 2 diabetic patients than those patients with AIN. Lm levels showed an inverse correlation with BMI ($r=-0.327$, $p=0.024$) and fasting blood glucose ($r=-0.412$, $p=0.05$). Lm was also positively correlated with scores for motor symptoms ($r=0.31$, $p=0.031$), weakness ($r=0.356$, $p=0.011$), wasting ($r=0.456$, $p=0.0009$), reflexes ($r=0.429$, $p=0.0019$), total motor ($r=0.426$, $p=0.002$), and total disability ($r=0.337$, $p=0.016$). Serum Lm concentration was also positively correlated with indices of neurotoxicity of sera in NIE-115 cell culture in diabetic patients ($r=0.379$, $p=0.035$). Lm levels in neuropathic patients with nontoxic sera were higher than in patients with toxic sera, but the difference bordered on statistical significance (659.18±46.2 vs. 564.60±30.6 ng/ml, $p=0.082$).

Conclusions: The differences in serum Lm levels between diabetic and AIN are most likely related to differences in metabolic and autoimmune pathogenetic factors. The close correlation between motor disability score and Lm suggests that Lm may be important for the integrity of large, myelinated motor fibers. Furthermore, the reduction in Lm levels in DN as opposed to AIN suggests a specific role in the neuropathy of diabetes. DN may thus derive from impaired Lm-mediated integrin signaling and constitute a prospective therapy.

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Morphological Validation of a Clinical Scoring System for Diabetic Polyneuropathy

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Background and Aims: Clinical scoring systems for diabetic sensorimotor polyneuropathy (DSP) range from the simple to complex, and are commonly referenced to a gold standard which is integral to the scoring system. Recently, a clinical scoring system (CSS) independent of an electrophysiological gold standard was used to determine severity of DSP in a cohort of subjects enrolled in a study of simple screening tests for DSP. The CSS successfully stratified subjects into non-neuropathic, and mild, moderate and severe DSP groups. Although electrophysiological results correlate with clinical scores and morphological changes, it is unknown how well clinical scores correlate with morphological changes. We aimed to determine whether the Toronto CSS reflects the morphological severity of DSP as indicated by changes in fibre density (FD) on sural nerve biopsy.

Materials and Methods: Ninety-six patients underwent comprehensive medical and neurological history and physical examinations; electrophysiological tests, quantitative vibration perception thresholds, and sural nerve biopsies. Application of the Toronto CSS determined a point value ranging from 0 to a maximum of 19 points for each subject. Correlations between the CSS value and myelinated FD were evaluated. Relationships between the FD and electrophysiological parameters were evaluated.

Results: The total clinical score showed a significant inverse correlation with the sural nerve FD ($R^2=0.256$, $p<0.0001$). The mean FD declined as the severity of CSS-defined DSP increased. Based on categorical HbA1C *8 compared to those >8, those with good control had a significantly lower ($p=0.0025$) total clinical score compared to those with poor control. Electrophysiological parameters showed significant correlations with morphological changes. Sural nerve FD inversely correlated with an amplitude score ($R^2=0.481$, $p<0.0001$) and a conduction velocity score ($R^2=0.515$, $p<0.0001$).

Conclusions: This study confirms the validity of the Toronto CSS in reflecting the presence and severity of DSP, and underscores the tight inter-relationships between clinical neuropathy, electrophysiological findings and morphological changes. The Toronto CSS may prove useful in documenting and following DSP in the clinic setting.

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GLYCAEMIC EXCURSIONS AND PAINFUL DIABETIC PERIPHERAL NEUROPATHY

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Background and Aims: Chronic sensory peripheral neuropathy affects 30% of type 1 diabetic patient. Some patients have painful symptoms whilst a majority are asymptomatic. The mechanism by which these disabling symptoms occur is poorly understood and the treatment of symptoms is often less than adequate. The aim of this study was to examine the relationship between glycaemic excursions and painful symptoms in patients with painful peripheral neuropathy.

Materials and Methods: Twenty type 1 diabetic patients with clinically significant peripheral neuropathy (10 painful and 10 painless) were asked to wear a MiniMed Continuous Glucose Monitoring System for three days. Patients with symptoms were asked to keep a record of painful episodes on a Visual Analogue Scale. We determined the Mean Amplitude of Glycaemic Excursions (MAGE), the M-value and percentage number of hypoglycaemic readings (<3.0 mmol/l) to assess glycaemic stability.

Results: Patients were matched for (mean ± SD) age: 52.0 ± 11.1 yrs; duration of diabetes: 24.8 ± 10.7 yrs; duration of neuropathy: 5.6 ± 2.6 yrs and number of glucose readings per 3-day period: 832 ± 87. The painful group had a greater mean glucose reading (12.1 ± 2.9 mmol/l vs. 9.3 ± 1.9 mmol/l, $p=0.02$) and a greater M-value (107.8 ± 51.3 vs. 53.8 ± 31.1, $p=0.02$), compared to the painless group. Additionally, the painful group showed a slight trend towards having more glycaemic excursions (5.0 vs. 3.0) and a greater percentage of hypoglycaemic readings (4.1% vs. 3.2%) over the 3-day monitoring period, but this did not reach statistical significance. There was no difference in the MAGE between the painful and the painless group (4.7 ± 1.9 mmol/l vs. 5.0 ± 1.9 mmol/l, $p=0.6$). In the painful group there was no significant correlation between the number of glycaemic excursions and the number of painful episodes.

Conclusions: Diabetic patients with painful neuropathy have poorer diabetic control, greater blood glucose flux and possibly more hypoglycaemic episodes, when compared to patients with painless neuropathy. Therefore, strict glucose regulation to improve glycaemic stability could be the best form of analgesia for painful diabetic neuropathy.

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 α -LIPOIC ACID ACCELERATES GALLBLADDER CONTRACTION ACTIVITY IN PATIENTS WITH DIABETIC CHOLECYSTOPARESISS.M.Tkach¹, O.P.Klimenko², ¹Institute of Endocrinology and Metabolism, Kyiv; ²Institute of Pediatrics, Obstetrics and Gynecology, Kyiv, Ukraine

Background and Aims: Reduced gallbladder contraction is one of the risk factors in cholesterol gallstones. Impaired gallbladder motility is associated with diabetic autonomic neuropathy. α -lipic acid has a positive effect on diabetic cardiovascular autonomic neuropathy. The aim was to estimate the efficacy of α -lipoic acid in patients with diabetic cholecystoparesis. **Subjects and Methods:** Thirty four type 1 diabetic patients (age: 26.0 ± 2.5 yrs; mean \pm SEM) with diabetic autonomic neuropathy adequately controlled with insulin were randomly divided into two groups. Twenty patients received α -lipoic acid, 600 mg once daily for 20 days. The other 14 sex and age matched patients represented the control group. We evaluated contraction activity of the gallbladder using real-time ultrasonography and function of autonomic nervous system by short-term spectral analysis of heart rate variability. **Results:** The patients treated with α -lipoic acid, unlike the control group, had a positive dynamics of clinical symptoms of gastrointestinal autonomic neuropathy and an increase in gallbladder ejection fraction at 30, 40, 60 min after egg yolk from $14.2 \pm 9.7\%$, $32.9 \pm 7.3\%$, and $27.6 \pm 12.6\%$, before to, respectively, $43.3 \pm 7.6\%$, $54.1 \pm 7.0\%$, and $64.8 \pm 6.6\%$, after treatment ($P < 0.05$). The residual gallbladder volume decreased from 20.4 ± 4.4 cm³ to 9.4 ± 2.8 cm³ ($P < 0.05$), versus control 20.4 ± 6.4 cm³ and 17.0 ± 5.5 cm³ ($P > 0.1$), respectively, before and after treatment. An increase in gallbladder motility after α -lipoic acid, in contrast to the control group, occurred with an increase in spectral power heart rate components: VLF – from 22.7 ± 4.3 ms² to 87.2 ± 22.1 ms² ($P < 0.02$), LF – 2.4 ± 0.5 ms² to 9.0 ± 2.7 ms² ($P < 0.05$), and a tendency to a rise in HF from 12.1 ± 2.6 ms² to 27.1 ± 7.2 ms² ($0.05 < P < 0.1$). **Conclusions:** α -lipoic acid had a positive effect on the gallbladder contraction in patients with diabetic gastrointestinal autonomic neuropathy.

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Lipids in Cardiovascular Complications

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Type 2 diabetic subjects with ischaemic heart disease: Postprandial lipaemic hyperresponders?

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Background and Aims: Postprandial lipaemia seems to play an important role for the development of coronary heart disease through an elevation of triglyceride-rich lipoproteins, e.g. chylomicrons (CM), very low density lipoproteins (VLDL), and remnant particles. Our aim was to compare postprandial metabolic profiles in two groups of type-2 diabetic patients, i.e. with and without ischaemic heart disease, respectively.

Materials and Methods: 32 male type-2 diabetics were included in the study. We matched 17 cases with a verified history of acute myocardial infarction with 15 controls according to age, Body Mass Index, glycated haemoglobin, diabetes duration, smoking, and treatment of diabetes. The only anti-hyperglycaemic treatment accepted was diet, Acarbose, and/or sulfonylurea. The patients underwent a hyperinsulinaemic, euglycaemic clamp to measure the insulin sensitivity. Furthermore, we studied the postprandial lipaemic responses. After an overnight fast, the patients took an oral fat load, consisting of 150 ml soup with 100 g butter, 25 g raw leek, and 94 g white bread. Total energy content was 4297 kJ with 76.6% of energy as fat and 20.1% as carbohydrate. 30 mg vitamin A was taken with the first spoonful of soup as a marker of lipoproteins of intestinal origin. During the next eight hours blood samples were drawn for the analysis of glucose, insulin, and triglyceride in total plasma, in a CM-fraction ($Sf > 1000$) and in a non-CM fraction (VLDL-rich fraction) ($Sf < 1000$).

Results: Insulin sensitivity was similar in the case- and control groups with M-values 3.6 ± 1.8 vs. 4.9 ± 3.2 mg glucose/(kg x min) ($p = 0.40$), respectively. No differences between the two groups were found in responses measured as incremental Area Under the Curve (AUC) for glucose ($p = 0.2$) or insulin ($p = 0.33$). Plasma TG levels in the case group were significantly higher after 360 minutes (4.6 ± 3.1 vs. 2.8 ± 1.8 mmol/L, $p = 0.04$) and 480 minutes (3.6 ± 2.2 vs. 2.4 ± 2.4 mmol/L, $p = 0.03$), as were the iAUC for the whole period (560 ± 452 vs. 297 ± 214 mmol/L x min, $p = 0.048$). Furthermore, the retinyl palmitate responses in the CM-fraction from the case-group were significantly higher with an iAUC of 311502 ± 194933 vs. 187004 ± 102928 ng/mlxmin, $p = 0.035$. No differences were found in the retinyl palmitate responses in the non-CM fractions ($p = 0.4$).

Conclusions: Type-2 diabetic subjects with prior myocardial infarction had impaired clearance of postprandial triglyceride-rich lipoproteins. Impaired clearance of postprandial chylomicrons might be a candidate risk marker for the development of ischaemic heart disease in type-2 diabetics.

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W-3 POLYUNSATURATED FATTY ACIDS IN THE TREATMENT OF CARDIOVASCULAR AUTONOMIC NEUROPATHY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Background and Aims: The aim of this study was to assess the long term effect of docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) on the heart rate variability (HRV), standart cardiovascular autonomic tests and QTc interval parameters, the activities of protein-kinase C (PK-C), Na⁺, K⁺-ATPase, Ca²⁺, Mg²⁺-ATPase, levels of fatty acids in the membranes of erythrocytes and the state of the prostacyclin 12-thromboxane A2 system in patients with Type 2 diabetes mellitus and cardiovascular autonomic neuropathy (CAN). **Materials and Methods:** CAN assessed by reduced HRV, standart cardiovascular autonomic tests and QTc interval parameters. 42 patients (54 \pm 5 years, 25m/17f) were allocated into two treatment groups. The 1st group (n=23) was receiving capsules of fish oil every day (2.0 g EPA, 2.0 g DHA and 0.1% α -tocopherol acetate) and the 2nd group (n=19) was receiving placebo capsules of olive oil. All patients were on the same diet. **Results:** After 2 months of treatment there was a decreasing of 12SI-thromboxane B2 (TXB2) level (152.5 ± 16.9 pg/ml, $p < 0.001$), activity of PK-C (12.37 ± 4.11 pmol 32P/mg protein per 1 min, $p < 0.001$) with simultaneous increasing of EPA level, EPA/arachidonic acid ratio, activities of Na⁺, K⁺-ATPase (from 0.04 ± 0.003 to 0.09 ± 0.004 mNmole Pi/mg protein per 1 hour, $p < 0.001$), Ca²⁺, Mg²⁺-ATPase and the level of the 12SI-6-ketoprostaglandin F1 α in the blood plasma in the first group. Also, we observed significant improvement of cardiovascular autonomic tests, HRV parameters, decreasing of QTc interval ($p < 0.01$). Increasing of the level of EPA, EPA/arachidonic acid ratio and the activities of membrane-bound enzymes lead to increasing of RBC's deformability. This effect is conditioned also by increased prostacyclin 12 production as well as by inhibition of platelet activity. Therefore it seems that a 4.0 g fish oil treatment during following 2 months result the tendency of normalizing the state of prostacyclin 12-thromboxane A2 system, activity of membrane-bound enzymes, HRV, QTc parameters and cardiovascular autonomic tests. **Conclusions:** In conclusion, DHA and EPA at moderate doses may exert antithrombotic effects and may be used for effective prophylaxis and treatment of diabetic cardiovascular autonomic neuropathy.

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POSTPRANDIAL LIPIDAEMIA AND LDL SIZE IN TYPE 2 DIABETES MELLITUS.

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Background and aims: Small LDL particles and postprandial hypertriglyceridaemia are considered components of diabetic dyslipidaemia. Our aim was to assess postprandial lipidaemia and LDL size in normolipidaemic non-obese type 2 diabetic patients, and find predictors of both.

Materials and methods: 12 type 2 non-obese, normotriglyceridaemic diabetic patients (8 male, BMI 25.77 ± 2.64 Kg/m², age 52.42 ± 10.36 years, HbA1c $6.80 \pm 0.67\%$) on diet, and 14 control subjects (8 male, 24.31 ± 2.40 Kg/m², age 54.0 ± 6.14 years) with similar fasting triglyceride were selected. A test meal (55g fat, 100.000 IU vitamin A) was given. LDL cholesterol (LDLc, betaquantification), HDL cholesterol (HDLc, direct) and apolipoprotein B (immunoturbidimetry) were measured in the fasting state. Triglyceride (Tg), retinylpalmitate (RP, HPLC) and LDL size (electrophoresis) were measured before and 2,3,4,5,6 and 8 hours after the meal. Lipoprotein (Lpl) and hepatic (HL) lipase activities were measured (artificial substrate, radiolabelled trioleate) on a separate day, after a heparin injection (100 IU/Kg).

Results: Patients showed lower fasting HDLc (1.12 ± 0.26 vs 1.40 ± 0.28 , $p = 0.016$) and smaller LDL particles 4 hours after the meal (25.86 ± 0.40 vs 26.16 ± 0.30 , $p = 0.041$). No differences were found in the area under the curve of Tg (AUC-Tg) or RP. Lpl was similar, but HL activity was higher in patients than in controls (156.63 ± 23.89 vs 118 ± 43.27 , $p = 0.011$).

Tg at 4 and 5 hours after the meal were the best predictors of AUC-Tg ($r = 0.944$ and 0.949 , respectively, $p < 0.01$). LDLc/apoB ratio was the best predictor of LDL size in the fasting state. Not only fasting LDL size ($r = 0.848$, $p < 0.0005$), but also fasting LDLc/apoB ratio and HL activity were good predictors of LDL size at 4 hours postprandially ($r = 0.520$, $p < 0.0005$ and $r = -0.442$, $p < 0.05$, respectively).

Conclusions: This group of well-controlled, non-obese normolipidaemic type 2 diabetic patients, with normal postprandial triglyceridaemia, shows low postprandial LDL size, associated with increased hepatic lipase activity. LDLc/apoB ratio is a good predictor of both fasting and postprandial LDL size.

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REPAGLINIDE REDUCES POSTPRANDIAL CHYLOMICRON-DEFICIENT PLASMA TRIGLYCERIDES IN PATIENTS WITH TYPE 2 DIABETES

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Background-Aims: Postprandial lipaemia is important in atherogenesis in both diabetic and non-diabetic subjects. The aim of this study was to investigate the effect of a single dose of repaglinide (R) on postprandial triglyceridaemia in patients with type 2 diabetes. **Materials and Methods:** After an overnight fast, 20 diabetic subjects [mean (SD) age: 57 (8.9) years], who had acceptable diabetes control with diet alone (HbA_{1c} < 8%), consumed a standard meal (485 Kcal: carbohydrates 40%, lipids 45%, proteins 15%) on two separate days: once with placebo (P) and once with 2 mg R per os in a blind order. Venous blood samples were drawn just before, and 1h, 2h, 4h and 6h after meal consumption. Triglycerides contained in chylomicrons (CMT) and in CM-deficient plasma (CMDT) were determined by ultracentrifugation. In addition, plasma total triglycerides (TR), total cholesterol (CHOL), HDL-cholesterol (HDL), LDL-cholesterol (LDL), free fatty acids (FFA), glucose, insulin and C-peptide levels were also measured. **Results:** As expected, there was a significant reduction in postprandial glycaemia after R administration compared to P [mean AUC (SD): 33.8 (7.8) vs 45.8 (13.4) mmol/l^{1-6h} respectively, P=0.0007]. AUC for insulin and C-peptide were significantly higher after R administration than P [472.2 (414.2) vs 247.7 (168.9) IU·ml^{-1-6h} respectively, P=0.01 and 50.9 (36.9) vs 32.4 (22.5) ng·ml^{-1-6h} respectively, P=0.03]. TR and CMT showed a trend to be lower after R compared to P but the difference was not significant. However, a significant reduction in postprandial CMDT was noted after R compared to P [AUC: 42.9 (13.4) vs 46.3 (14.4) mmol/l^{1-6h} respectively, P=0.05]. Postprandial levels of CHOL, HDL, LDL and FFA were not significantly different between the two meals. **Conclusions:** Acute administration of repaglinide reduces postprandial triglyceride levels of liver-but not of intestinal-origin in patients with type 2 diabetes. This effect might be due to either the substantial improvement of postprandial glycaemia after R or to the drug-induced insulin secretion per se.

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SERUM PEROXONASE ACTIVITY AND LIPID PEROXIDATION IN TYPE 2 DIABETIC WOMEN WITH DIFFERENT MENOPAUSAL STATUS

M. Gorshunskaya and O. Bilezka. Kharkiv Postgraduate Medical Academy, Ukraine. **Background and Aims:** Women with diabetes are denied estrogens-associated cardioprotection. In view of accumulating evidence as to the role of oxidative stress and damage to lipoproteins in atherogenesis we evaluated the impact of menopausal status on lipid peroxidation parameters, lipid profile and antioxidant defence in Type 2 diabetic women (DW). **Materials and Methods:** 78 pre-, peri-, or postmenopausal DW 35 to 65 years old were examined (duration of diabetes: 6.9±0.7 yrs; fasting blood glucose: 5.1±0.1 mmol/L, HbA_{1c}: 6.0±0.2%; BMI: 31.2±0.7 kg/m²). 21 healthy, sex-, age matched subjects acted as controls. Peroxonase (PON), HDL-associated enzyme capable of hydrolysing lipid peroxides, was assessed using paraoxon as a substrate. HOMA algorithm was employed to evaluate insulin resistance (IR). **Results:** Compared with controls DW had significantly (p<0.001) higher values for serum triglyceride (TG), total cholesterol (TC), malonic dialdehyde (MDA), conjugated dienes (CD), preformed hydroperoxides (HPO) contents in VLDL+LDL and HDL and HOMA-IR indices. Serum HDL-C, PON activity and (VLDL+LDL)- and HDL-α-tocopherol (TPH) contents were lower than in controls (p<0.001) in diabetics by 32, 33, 39 and 60 %, respectively, with no differences observed between pre-, peri-, and postmenopausal DW. Strong negative relationship (p<0.001) was revealed between PON activity and indices of lipid peroxidation, i.e. CD (r=-0.687), (VLDL+LDL)- and HDL-HPO (r=-0.478, r=-0.472, respectively). PON activity was significantly (p<0.01) inverse correlated with MDA (r=-0.413), TC (r=-0.352) and LDL-C (r=-0.362) levels at the same time as it was directly associated with (VLDL+LDL)-α-TPH (r=0.442, p<0.001) and HDL-α-TPH (r=0.408, p<0.01). **Conclusions:** Thus, despite good glycemic control Type 2 diabetic women with insulin resistance show reduced antioxidant defence, in particular, serum paraoxonase activity, and augmented lipid peroxidation in the lack of modulatory effect of menopausal status on these parameters.

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THE EFFECTS OF OESTROGENS ON POSTPRANDIAL LIPID METABOLISM IN WOMEN WITH TYPE 2 DIABETES

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Background and Aims: Women with type 2 diabetes (DM) lose the protection against cardiovascular disease (CVD) afforded by oestrogens, compared to healthy controls. We have investigated the effects of oestrogen replacement on postprandial lipid metabolism in women with DM. **Materials and Methods:** Postmenopausal diabetic subjects on and off hormone replacement therapy (HRT) were contrasted with matched healthy controls. Subjects consumed a mixed meal labeled with 1,1,1-¹³C-tripalmitin, with blood sampled over the next 6 hours. Concentrations of plasma triglyceride (TAG), non-esterified fatty acids (NEFA) and ¹³C-palmitic acid (¹³C-PA) in the TAG and NEFA fractions were measured over the next 6 hours using gas chromatography-combustion-isotope ratio mass spectrometry. **Results:** (median (range)) In the control groups, HRT did not affect fasting TAG (C-HRT vs C+HRT 1.2 (0.5-2.7) vs 1.0 mmol/l (0.4-1.8), p=0.15) or NEFA (C-HRT vs C+HRT 120 (84-158) vs 152 (91-183) mmol/l, p=0.12), but did improve postprandial TAG metabolism (TAG area under the curve (AUC) C-HRT vs C+HRT 9.7 (4.7-18.5) vs 7.7 mmol/l per 6h (4.1-12.8), p=0.018; ¹³C-PA AUC 41.1 (15.5-77.2) vs 25.0 ug/ml per 6h (10.3-47.7), p=0.05). NEFA ¹³C-PA rose due to HRT (C-HRT vs C+HRT AUC 1.9 (1.2-2.6) vs 2.5 (1.6-3.5) mmol/l per 6h, p=0.046). There was no difference between diabetic groups in fasting TAG, TAG AUC, TAG ¹³C-PA AUC, fasting NEFA, or NEFA ¹³C-PA AUC (DM-HRT vs DM+HRT: fasting TAG 2.3 (0.6-5.8) vs 2.7 mmol/l (1.2-4.8), p=0.40; TAG AUC 16.9 (5.8-40.8) vs 15.8 mmol/l per 6 hours (6.8-30.7), p=0.75; TAG ¹³C-PA AUC 51.5 (13.5-121.6) vs 39.9 ug/ml per 6 hours (18.8-59.8), p=0.18; fasting NEFA 176 (129-327) vs 172 (144-220) mmol/l, p=0.46; NEFA ¹³C-PA AUC 2.1 (0.9-6.5) vs 2.3 (1.7-3.4) mmol/l per 6h, p=0.12). **Conclusions:** Oestrogens improve postprandial lipid metabolism in healthy postmenopausal women but in type 2 diabetes, independently of fasting lipid levels. This may affect HDL and LDL metabolism, contributing to CVD in women with DM.

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EFFECTS OF ATORVASTATIN VS GEMFIBROZIL ON DIABETIC DYSLIPIDAEMIA.

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Background and Aims: Both statins and fibrates are useful in the treatment of diabetic dyslipidaemia. Our aims were to compare their effect on lipoproteins and LDL size in type 2 diabetes.

Materials and methods: A group of 44 type 2 diabetic patients (age 57.7 ± 9.0 years, BMI 27.8 ± 3.5 Kg/m², HbA_{1c} 6.9 ± 0.6 %) with LDL cholesterol (LDLc) > 2.6 mmol/l and triglyceride (Tg) < 5.65 mmol/l were included in an open, randomised, cross-over study comparing 12-week treatment with 900-1200 mg gemfibrozil vs 10-20 mg atorvastatin, separated by a 4-week wash-out period. Total cholesterol (TC) and Tg, HDL cholesterol (HDLc, direct method), LDLc (Friedewald/ultracentrifugation) LDL size (gradient gel electrophoresis) and apolipoprotein B (apoB, immunoturbidimetry) were measured at baseline and after intervention. **Results:** At baseline, TC was 5.8 ± 0.8, Tg 1.7 ± 1.0, LDLc 3.8 ± 0.5, HDLc 1.1 ± 0.3 mmol/l, apoB 1.2 ± 0.1 g/l, and LDL size 25.6 ± 0.5 nm (41% showed phenotype B), without difference between groups starting with gemfibrozil or atorvastatin. Atorvastatin and gemfibrozil significantly reduced Tg (-0.24 ± 0.10 vs -0.51 ± 0.10 mmol/l, respectively, p=0.068 between groups), and increased HDLc (0.040 ± 0.02 vs 0.039 ± 0.02 mmol/l, p 0.94 between groups). Atorvastatin was more effective in decreasing LDLc (-1.28 ± 0.08 vs -0.18 ± 0.08 mmol/l, p<0.0001), LDLc/HDLc ratio (-1.17 ± 0.08 vs -0.23 ± 0.08, p<0.0001) and apoB (-0.30 ± 0.02 vs -0.07 ± 0.02 g/l, p<0.0001). Gemfibrozil was more effective in reducing VLDLc (-0.10 ± 0.04 vs -0.24 ± 0.04, p 0.012), and it increased LDL size (0.22 ± 0.07 nm, p 0.0041 vs baseline, but p=0.057 between groups).

Conclusions: Both atorvastatin and gemfibrozil are effective, and complementary, in the treatment of diabetic dyslipidaemia.

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FENOFIBRATE GENERATES GREATER INCREASES IN LDL SIZE THAN ATORVASTATIN IN DYSLIPIDEMIC PATIENTS UNDER TREATMENT FOR 12 WEEKS.

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Background and Aims: The presence of small, dense LDL particles, one of the features of the high triglyceride-low HDL-cholesterol dyslipidemia linked to abdominal obesity and the insulin resistance syndrome, is predictive of an increased risk of coronary heart disease. The objective of the present study was to compare the effects of a 12-week pharmacotherapy with micronized fenofibrate (200 mg) or atorvastatin (10 mg) on LDL particle size of dyslipidemic patients.

Materials and Methods: After a 4-week washout period, dyslipidemic patients (HDL-cholesterol <1.1 mmol/l or <1.2 mmol/l for men and women respectively, LDL-cholesterol >3.2 mmol/l and triglycerides <4.5 mmol/l) were randomized to either fenofibrate (n=64) or atorvastatin (n=72) therapy for 12 weeks. LDL particle size measurement was performed by 2-16% polyacrylamide gradient gel electrophoresis on frozen plasma.

Results: Both fenofibrate and atorvastatin therapies increased HDL-cholesterol levels (+12.7% vs. +5.7%) and decreased triglyceride concentrations (-29.4% vs. -15.4%). Whereas improvements in the plasma lipoprotein-lipid profile were observed with both fenofibrate and atorvastatin drugs, the increase in LDL peak particle diameter was significantly greater among fenofibrate- than atorvastatin-treated patients (4.92 ± 3.31 vs. 1.75 ± 2.87 Å; $p < 0.0001$). Moreover, significant relationships were found between changes in triglycerides and changes in LDL peak particle size in both fenofibrate ($r = -0.40$, $p < 0.001$) and atorvastatin ($r = -0.48$, $p < 0.0001$) groups.

Conclusions: It is proposed that this effect of fenofibrate therapy on LDL size is one factor involved in the favorable impact of fibrates on the recurrence of coronary heart disease events.

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LDL-Immune Complexes in Type 2 Diabetes and Vascular Disease

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Background and Aims: The oxidative modification of LDL has been shown to affect its clearance and exert cytotoxic and immunogenic effect. The objective of our study was to analyse markers of LDL oxidation i.e. soluble LDL containing immune (LDL-IC) complexes in type 2 diabetes with micro- and macrovascular disease.

Materials and Methods: We recruited 69 diabetic patients with coronary artery disease (DM+CAD), 78 nondiabetics with CAD, 47 controls, 27 diabetics with nephropathy and 36 free of complications. OxLDL antibodies and advanced glycosylated endproducts were measured by ELISA and LDL-IC apo B content after PEG precipitation.

Results: We measured a broad range of oxLDL antibody activity in all study groups, but without significant differences. In contrast, the content of apo B, a component of the antigen moiety of oxLDL-IC was higher in CAD and diabetes (+CAD) than that in LDL-IC isolated from controls ($p < 0.001$). LDL-IC did not differ between patients with CAD+DM and CAD patients free of diabetes. LDL-IC level in diabetic patients with or without microangiopathy was significantly higher than that in healthy volunteers (PEG-apo B: 0.278 ± 0.107 vs. 0.165 ± 0.105 g/l, $p < 0.002$; PEG-IgG: 151.7 ± 76 vs. 115.4 ± 62 g/l, $p < 0.05$). However, we did not find a significant difference in the quantity of circulating LDL-IC between the subgroup of diabetic patients with nephropathy/retinopathy and the patients free of microvascular disease (Ab-oxLDL 27.7 ± 10.4 vs. 27.1 ± 9.3 AU, NS; PEG-apoB 0.324 ± 0.111 vs. 0.287 ± 0.124 g/L, NS; PEG-IgG 1.68 ± 0.68 vs. 1.42 ± 0.80 g/L, NS). The correlation between AGE content and LDL-IC was positive and statistically significant ($r = 0.35$, $p < 0.009$). We observed a significant but inverse correlation between triglyceride concentration and the amount of LDL-IC in diabetes with CAD ($r = -0.32$, $p < 0.016$) and in CAD patients ($r = -0.35$, $p < 0.002$). In patients with early nephropathy a very significant negative correlation between triglycerides and circulating LDL-IC ($r = -0.54$, $p < 0.039$) was found, but not so in patients with physiological proteinuria. It is known that at high triglyceride level in type 2 diabetes, the majority of LDL is small and dense, thus being more susceptible to oxidative modification. This could be a possible mechanism explaining why more LDL-IC, with a level inversely correlating with triglyceride concentration, are generated in diabetes. **Conclusions:** Increased level of circulating LDL-IC represent a general risk factor for the general population, including diabetes. LDL-IC measurements in type 2 diabetes plus CAD, with or without microangiopathy showed that diabetic patients are a high-risk population.

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FENOFIBRATE GENERATES GREATER INCREASES IN HDL-CHOLESTEROL LEVELS THAN ATORVASTATIN IN THE TREATMENT OF OBESE AND NONOBESE DYSLIPIDEMIC PATIENTS

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Background and Aims: A low HDL-cholesterol (HDL-C) concentration which is a risk factor for coronary heart disease is frequently found in obese patients. The objective of the present study was to compare the HDL-C raising effects of micronized fenofibrate (200 mg) vs. atorvastatin (10 mg) in nonobese and obese dyslipidemic patients.

Materials and Methods: After a washout period of 4 weeks, dyslipidemic patients [HDL-cholesterol below 1.2 mmol/l (women) and 1.1 mmol/l (men) with LDL-cholesterol above 3.2 mmol/l and triglyceride levels < 4.5 mmol/l] were randomized to receive either fenofibrate (n=79) or atorvastatin (n=86) once daily for 12 weeks.

Results: Overall, atorvastatin therapy increased HDL-C levels by 5.3 % compared to a 13.3 % increase in the fenofibrate-treated patients with a difference of 8 % [95% CI: 3.7% to 12.2%] ($p = 0.0003$). Patients were then stratified on the basis of their baseline body mass index (BMI) using 30 kg/m² (according to WHO guidelines). Among obese (BMI > 30 kg/m²) dyslipidemic patients, atorvastatin increased HDL-C concentrations by 5.3% [95% CI: 0.5% to 10.1%] whereas fenofibrate generated an increase of 11.0% [95% CI: 6.2% to 15.8%] in HDL-C levels. Similar differences in the response of HDL-C between atorvastatin and fenofibrate were observed in nonobese (BMI < 30 kg/m²) dyslipidemic patients.

Conclusions: These results suggest that fenofibrate is better than atorvastatin to raise HDL-C levels in both nonobese and obese dyslipidemic patients with low HDL-C levels.

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EFFECT OF CERIVASTATIN ON MUSCLE CAPILLARY FILTRATION OF ALBUMIN IN DIABETIC RATS.

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Background and aim: An increase in capillary filtration is a well-known complication of diabetes. Some data suggest that statins are beneficial for endothelium function. The aim was to examine the effect of cerivastatin on capillary filtration of albumin (CFA) in rats with streptozotocin-induced diabetes. **Materials and methods:** Fourty STZ rats, aged 82 ± 6 days were randomized in two groups, treated by cerivastatin (group 1), or untreated (group 2). CFA was evaluated in vivo by a reproducible noninvasive isotopic test which consisted of injecting i.v 99m-Tc albumin and measuring radio-activity externally with a gamma camera on a posterior limb, before, during and after venous compression. The test was performed before randomization (T0), 2 months (T1) and 3 months (T2) later. **Results:** In both groups, interstitial albumin retention after removal of venous compression (AR) was significantly higher than in age-matched control rats (group 1: $2.4 \pm 1.0\%$ and group 2: $6.7 \pm 1.3\%$, vs $0.68 \pm 0.32\%$; $p < 0.001$). In group 1, AR did not change at T1 ($3.0 \pm 1.5\%$) and T2 ($3.0 \pm 0.9\%$), while in group 2, AR increased at T1 ($17.0 \pm 3.1\%$) and T2 ($27.7 \pm 3.0\%$). The differences between groups 1 and 2 were significant at T1 and T2 ($p < 0.005$ and $p < 0.001$). The lymphatic uptake of interstitial albumin evaluated by LF/HF index from fast Fourier Transform of the decreasing radio-activity curve after removal of venous compression did not differ at T0 between STZ and control rats. LF/HF increased slightly in groups 1 and 2 at T1. At T2, LF/HF was significantly lower in group 1 than in group 2 ($0.23 \pm 0.05\%$ vs $2.2 \pm 0.6\%$; $p < 0.007$). **Conclusion:** These data show that the increase in CFA occurs early and worsens with time in diabetic rats, while lymphatic dysfunction occurs later, probably as a result of saturation of lymphatic pumps. Both alterations are prevented by cerivastatin. This microcirculatory effect of cerivastatin on endothelial function is very likely to be independent of its lipid-lowering effect.

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Diabetes and the Heart

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ADMISSION BLOOD GLUCOSE, IRRESPECTIVE OF DIABETES STATUS, PREDICTS SURVIVAL FOLLOWING MYOCARDIAL INFARCTION (MI)

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Background & Aims: Although admission blood glucose (BG) is known to correlate with in-hospital mortality following acute MI, the relationship between BG on admission and longer term survival in diabetic and non-diabetic subjects, is unclear. We have investigated this relationship in Southern Derbyshire (population > 500,000) in patients admitted with acute MI during a 4-year period 1995-8.

Methods: A single pathology service linked to all acute hospital admissions and recorded deaths. Patients with an ICD10 code for acute MI were linked to their laboratory data for the admission.

Results: Of 2404 pts coded for first MI, 1,858 (77%) of these patients, including 280 (15%) with known diabetes (DM), had documented BG levels on admission. Mortality at 30-days and 1-yr was significantly higher among those with admission BG > 11mmol/L (Table), and the continuous relationship between admission BG and mortality was irrespective of known DM status.

	DM BG>11	DM BG<11	NO DM BG>11	NO DM BG<11
30-d mortal. (%)	34.6	31.7	31.3	16.9
1-yr mortal. (%)	40.8	44.3	41.9	24.5

Adm. BG (n)	>11 (466)	10.1-11 (89)	8.1-10 (327)	6.1-8.0 (620)	≤6 (346)
30-d mortal.	33%	34%	23%	14%	16%
1-yr mortal.	42%	35%	32%	22%	23%

Conclusion: Admission BG level, independent of known DM status, is a powerful predictor of survival both at 30-days and 1-yr after AMI.

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SECONDARY PREVENTION MEDICATIONS IN DIABETIC PATIENTS AFTER AN ACUTE CORONARY SYNDROME.

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The French nation-wide PREVENIR studies were designed to assess prescriptions at hospital discharge following a myocardial infarction (MI) or an unstable angina (UA). The PREVENIR-1 survey included 1394 patients (MI: 55.8%) in 77 cardiology centers in January 1998 and the PREVENIR-2 Study included 2527 patients (MI: 65.6%) hospitalized in 143 centers in May and June 1999. A predominance of men (71.4% in 1998 and 74.2% in 1999) and subjects aged 65 yr or older (57.3% and 50.6%) were observed. The following analysis evaluated the impact of diabetic status on the secondary prevention medications. **Results:** The prevalence of diabetes mellitus, defined by insulin or oral hypoglycaemic agents prescriptions at discharge, was 17.4% in 1998 and 15.1% in 1999. Insulin-treated subjects represented 33.8% of the whole diabetic population in 1998 and 40.1% in 1999. The prescription rate of statins increased in the diabetic population from 33.5% (vs 35.3% in non-diabetics, ns) in 1998 to 56.0% (vs 59.7%, ns) in 1999. In diabetic patients, the prescription rate of beta-blockers was respectively 58.4% (vs 69.4%, p<0.01) in 1998 and 72% (vs 75.4%, ns), the prescription rate of ACE inhibitors was 50.4% (vs 39.7, p<0.05) in 1998 et 51% (vs 39.6%, p<0.001) in 1999, and the prescription rate of calcium channel inhibitors was 36.4% (vs 26.2%, p<0.001) in 1998 and 28% (vs 21.1%, p<0.01) in 1999. In multivariate analysis, diabetic status significantly influenced the beta-blockers (OR=0.7, CI 0.5-0.9), ACE inhibitors (OR=1.4, CI 1.01-1.9), and calcium channel blockers (OR=1.4, CI 1.03-1.9) prescriptions in 1998, but only ACE inhibitors prescription (OR=1.35, CI 1.06-1.7) in 1999. **Conclusion:** These data underline the improvement in statins and beta-blockers prescription rates in diabetic patients with a coronary heart disease, according to current recommendations.

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MYOCARDIAL FUNCTION AND HYPOLYCEMIC TREATMENT IN TYPE 2 DIABETES PATIENTS WITHOUT ISCHAEMIC HEART DISEASE

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Background and Aims: Type 2 diabetes mellitus is often associated with impaired myocardial function. Recently it has been suggested that some modalities of antidiabetic treatment might have infavourable impact on cardiovascular system in type 2 diabetic subjects. The aim of the study was to assess myocardial function in regard to various hypoglycemic therapies. **Materials and Methods:** The study group consisted of 78 normotensive type 2 diabetes subjects (mean age 56.2±7.5 years, mean diabetes duration 8.1±4.3 years, body mass index 27.7±4.1 kg/m²) with no clinical or electrographical signs of ischaemic heart disease. They were divided into three groups according to the hypoglycemic treatment applied: 1 - sulfonylurea and metformin (n=26); 2 - insulin only (n=27); and 3 - insulin and metformin (n=25). Each treatment modality was used for at least one year in each patient. All patients had the following examinations performed: resting and exercise electrocardiography (ECG), 24-hour Holter ECG monitoring, high frequency ventricular late potentials, heart rate variability (HRV) assessment, and stress, 2-D and tissue Doppler echocardiography. Fasting plasma insulin, C-peptide, glucose, lipids and HbA_{1c} were measured. **Results:** The poorest cardiovascular examinations results were found in group 3 patients (left ventricular end diastolic and systolic volume: 4.0 and 3.06 m/s² respectively, left ventricular compliance: 0.004 g/m², HRV: 194 m/s², transient ischemic events: 3.2 per patient, ventricular late potentials: 10%; p<0.05 vs group 1 and 2), whereas there were no statistical significant differences in metabolic control of diabetes or duration of the disease between the studied groups. In particular, overall HbA_{1c} was 7.6±0.9%, fasting plasma glucose 145±29, total cholesterol 200±33, HDL cholesterol 47±7, triglycerides 136±68 mg/dl. **Conclusions:** Based upon our results, it may be suggested that left ventricular dysfunction and cardiovascular autonomic regulation impairment might be related not only - as it is believed - to the level of metabolic control of diabetes and the duration of the disease, but also to the type of hypoglycemic treatment used. However, the confirmation of this somewhat surprising finding requires further studies.

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ANGIOGRAPHIC DATA OF CORONARY ATHEROSCLEROSIS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS.

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Background and Aims: Prevalence and incidence of coronary heart disease are increased in patients with type2 (non-insulin-dependent) diabetes mellitus. The purpose of this study was to investigate the prevalence of coronary arteries (CA) stenosis in patients suffering from coronary artery disease (CAD) with and without diabetes mellitus. **Materials and Methods:** We studied 140 patients with CAD - 30 with type 2 diabetes mellitus (mean age was 53.3±6.7 years, data presented as mean±SD) and 110 without diabetes (aged 52.6±10.1 years). The CA were evaluated using selective coronaryangiography. The results were compared using Student's paired test and x²-test where appropriate. **Results:** The BMI was higher in patients with diabetes compared to control subjects - 31.7±6.3 and 25.0±2.3 kg/m², respectively, p<0.05. However, in those with diabetes it was significant increase of triglycerides levels - 2.6±1.6 vs. 1.8±0.9 mmol/l, p<0.05. The groups were comparable by total cholesterol, LDL and HDL levels, smoking, mean blood pressure and history of previous myocardial infarction. We found that numbers of CA with stenosis were higher in patients with diabetes - 2.7±0.9 vs. 1.9±0.8 arteries per patient in those with and without diabetes, respectively, p<0.001. In particular, the difference between the two groups was accounted for by a higher prevalence of three-vessel disease - 66.7% vs. 30.6% in diabetic and non-diabetic groups, respectively, p<0.001. The mean number of stenosis (either in the same or different CA) was 3.4±1.5 and 1.9±0.8 in patients with and without diabetes, respectively, p<0.005. Furthermore, in those with diabetes it was increased number of low grade stenosis (≤ 50% of vessel diameter) - 31.3 vs. 12.8%, respectively, p<0.05 and higher prevalence of total artery occlusion (100% of vessel diameter) in patients with diabetes - 39.4 vs. 26.5%, p<0.05. In contrast, in non-diabetic group it was increased number of more severe stenosis (>90% of vessel diameter) - 41.7 vs. 11.1%, respectively, p<0.05. Severe stenosis (≥75% of vessel diameter) were similar in both groups (19% non-diabetic vs 18.2% diabetic). **Conclusions:** Diabetes mellitus is associated with the higher prevalence of CA stenosis with the highest prevalence of low grade stenosis and total artery occlusions. These peculiarities of CAD could contribute to higher incidence of myocardial infarction in patients with diabetes mellitus.

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ANGIOGRAPHIC CHARACTERISTICS OF CORONARY ATHEROSCLEROSIS IN PATIENTS WITH DIABETES

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Background and Aims: Coronary atherosclerosis (CA) confers higher mortality in diabetic than in non-diabetic subjects. The mechanisms for this worse prognosis are unclear. The notion that diabetic patients develop more diffuse and more peripheral atherosclerosis could explain the poor outcome, but this pattern has been described rather in type 1 than in type 2 diabetes.

Materials and Methods: 757 consecutive patients referred to coronary angiography were investigated. Extent of CA was defined as the number of significant lesions ($\geq 50\%$), severity as the mean percentage of individual stenosis, diffuse sclerosis as nonfocal, nonsignificant irregularity of the coronary artery wall. According to glycemia patients were divided into 4 groups: established type 2 diabetes ($n=128$), fasting plasma glucose (FPG) > 125 mg/dl ($n=60$), HbA1c $> 6.1\%$ ($n=99$), and patients with none of the 3 criteria ("normoglycemic", $n=464$).

Results: Extent of CA was higher in the hyperglycemic patients than in the "normoglycemic" patients (1.56 vs. 1.50 vs. 1.63 vs. 1.30). It proved significantly increased in the pooled hyperglycemic subjects compared to the "normoglycemic" (1.57 vs. 1.30, $p=0.02$). The severity of CA was comparable in all four groups (79.77 vs. 79.89 vs. 76.46 vs. 78.52% mean stenosis), as was the prevalence of diffuse coronary sclerosis (70% vs. 75% vs. 65% vs. 66%).

Conclusions: Diabetic patients express a pattern of multifocal significant atherosclerotic lesions whereas the severity of CA is not affected by the glycemic status. Moreover, contrary to the current view, patients with type 2 diabetes do not have more diffuse coronary disease than non-diabetic patients with CA. In summary, CA in diabetic patients is specifically characterized by a significantly increased number of distinct focal lesions.

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DETECTING SILENT CORONARY STENOSES IN DIABETIC PATIENTS. EXERCISE STRESS TEST OR EXERCISE MYOCARDIAL SCINTIGRAPHY?

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Background and aims: Silent coronary stenoses (CS) are common and associated with poor prognosis in diabetic patients. The potential benefits of diagnosing CS include initiating anti-ischemic therapy and performing early revascularisation procedure. Myocardial scintigraphy (MS) is appropriate to detect silent CS but expensive. American (ADA) and French (ALFEDIAM) diabetes associations recommend to perform in that purpose ECG stress test (EST) at first line in diabetic patients with additional cardiovascular risk factors and able to exercise. The aim of the study was to determine if coupling MS to EST is effective to detect more patients with silent CS. **Materials and methods:** A total of 400 diabetic asymptomatic patients with ≥ 1 additional risk factor were consecutively recruited in our university department between January 1995 and June 2000 to undergo MS after pharmacological stress (dipyridamole injection) or, when they were able to exercise, maximal (85% of the maximum predicted heart rate reached) EST. The patients with abnormal results underwent coronary angiography. The 262 patients who underwent maximal EST coupled with MS were eligible for the study analysis. **Results:** The patients (150 males, 112 females) were 57.6 ± 8.8 years old, with diabetes (256 type 2) for 12.8 ± 7.7 years. EST results (clinical and electrical criteria) were abnormal in 54 patients, including 12 with associated abnormal MS results (imaging criteria). Of these, 43 underwent a coronarography, showing CS in 18 patients. Despite normal EST results, abnormal MS results were found in 42 additional patients. A coronarography was performed in 33 of them and exhibited CS in 16 patients. The predictive value of abnormal EST results alone ($n=42$), abnormal MS results alone ($n=42$) or both ($n=12$) to detect CS was respectively 34.4%, 48.5% and 63.6%. **Conclusion:** These findings in our large cohort suggest that exercise MS, which includes EST and MS, instead of EST alone should be the first-line testing to detect silent CS in diabetic patients able to exercise.

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DETERMINANTS OF SILENT MYOCARDIAL ISCHEMIA IN DIABETIC PATIENTS. INFLUENCE OF AGE AND DURATION OF DIABETES.

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Background and aims: Silent myocardial ischemia (SMI) is found in 20-30% of diabetic patients without cardiac symptoms. The aim of this multicenter study was to evaluate the influence of age and diabetes duration on the prevalence of SMI and silent coronary stenoses (CS) in diabetic patients, in order to improve the identification of patients who should benefit from cardiac investigations.

Materials and methods: SMI was assessed by myocardial scintigraphy combined with a stress test or dipyridamole administration or both, in 404 diabetic patients (50 type 1 and 354 type 2) without cardiac history but with at least two additional cardiovascular risk factors.

Results: SMI was found in 138 patients (34.1%). A coronary angiography was performed in 63 of them, showing significant CS in 37 cases. The positive predictive value of myocardial ischemia for the detection of CS was therefore 58.7%. The prevalence of SMI did not differ significantly according to type of diabetes, nephropathy, peripheral arterial disease but it was significantly higher in the patients older than 60 yrs ($p=0.026$). Among the 153 men older than 60 yrs or aged 40-60 yrs with a diabetes duration > 20 yrs, 81 had SMI and a coronary angiography was performed in 28 of them, showing significant CS in 23 cases. Among the 99 women older than 60 yrs or aged 50-60 yrs with a diabetes duration > 10 yrs, 26 had SMI and a coronary angiography was performed in 16 of them, showing CS in 11 cases. In this selected population of men and women, the predictive value of myocardial scintigraphy for CS was 82.1% and 68.7% respectively in men and women.

Conclusion: Among the diabetic patients with other cardiovascular risk factors, these criteria of selection made possible the identification of 77.5% of the patients with SMI and 91.9% of those with CS, with a markedly improved predictive value of myocardial scintigraphy to detect CS.

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DISTURBED GLUCOSE METABOLISM IS UNEXPECTEDLY COMMON IN PATIENTS WITH MYOCARDIAL INFARCTION

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Background and aims: Increasing B-glucose adds to the risk for coronary artery disease. The actual prevalence of impaired glucose tolerance (IGT) and diabetes mellitus (DM) in a population with acute myocardial infarction (AMI) and without previously known DM is, however, not known. Our aim was to characterise glucose metabolism in patients with AMI and without known DM.

Materials and Methods: Consecutive non-DM patients with AMI and an admission B-glucose (a-BG) < 11.1 mmol/l admitted to two coronary care units were included. The a-BG was determined as soon as possible and fasting B-glucose (f-BG) and HbA1c on the first morning. An oral glucose tolerance test (OGTT) was performed before hospital discharge and 3 months thereafter.

Results: Inclusion criteria were fulfilled by 181 pat (age 63 ± 9 years; males 68%). The mean a-BG and HbA1c were 6.5 ± 1.4 mmol/l and $5.0 \pm 0.6\%$ respectively. On the OGTT at discharge in all 41% had IGT and 24% had diabetic values. After 3 months 48% had IGT and 22% DM. Thus, a total of 70% of the patients had disturbed glucose metabolism 3 months after the index event. If only fasting B-glucose was used just 6% had impaired fasting glucose and 14% had DM after 3 months. There was a strong positive correlation between admission HbA1c and abnormal OGTT after 3 months ($r=0.405$; $p=0.0001$) suggesting a predictive value of HbA1c for previously unknown glucose abnormalities in acute coronary care.

Conclusions: We found that newly detected diabetes (22%) and IGT (48%) was unexpectedly common in an unselected AMI population without previously known DM. Furthermore these abnormalities could be detected early in the course of an AMI. Thus elevated glucose parameters in the acute phase of a myocardial infarction can not be only a stress phenomenon.

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PREVALENCE AND CAUSES OF LEFT VENTRICULAR HYPERTROPHY IN JAPANESE TYPE 2 DIABETIC PATIENTS WITHOUT HYPERTENSION

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BACKGROUND AND AIMS: We investigate the prevalence of left ventricular hypertrophy (LVH) and clinical or genetic risk factors for development of LVH in Japanese type 2 diabetes without hypertension.

MATERIALS AND METHODS: M-mode echocardiography was performed by one experienced examiner in 98 consecutive, normoalbuminuric (albumin creatinine ratio (ACR) <200 mg/g Cr) type 2 diabetic patients with blood pressure <140/90 mmHg. Due to technical difficulties two patients could not be evaluated, thus 96 (63 men) patients were included in the study: age (mean (SD)) 56 (9) years, known duration of diabetes 11 (range 1-29) years. Body mass index (BMI) was 23 (3) kg/m², HbA1c 7.3 (0.9) %, blood pressure 122 (10) / 76 (7) mmHg, ACR (median (range)) 6.5 (2.4-120) mg/g Cr (10% microalbuminuria) and 61 (64%), 27 (28%), 11 (11%) had nil, simple, proliferative retinopathy, respectively. Angiotensin-converting enzyme (ACE) gene insertion (I) / deletion (D) polymorphism was determined by polymerase chain reaction.

RESULTS: The prevalence of LVH was 23% (95% CI 14-32%). LVH was present in 9 (27 (12-43)%) women and 13 (21 (11-31)%) men. Patients with LVH had higher BMI ($p=0.02$) and elevated systolic blood pressure ($p=0.03$) as compared to patients with normal left ventricular mass index (LVMI). There was no significant difference between the patients with LVH and the patients with normal LVMI regarding age, HbA1c, ACR, diastolic blood pressure, known duration of diabetes or presence of retinopathy. A logistic regression analysis, presence or absence of LVH, revealed that BMI and systolic blood pressure were independent risk factors for LVH ($p<0.05$, $R^2=24\%$). Patients with the DD genotype of the ACE gene had a higher LVMI (115 (95%CI: 103-127) g/m²) than those with ID (102 (93-110) g/m²) or II (94 (85-103) g/m²) genotype, ($p=0.02$).

CONCLUSION: Our study shows that LVH is frequent in Japanese type 2 diabetes without hypertension. Slightly increased BMI and systolic blood pressure may affect hypertrophic myocardial reactivity, and the DD genotype appears to be an important factor to predict increased LVMI for normotensive Japanese type 2 diabetes.

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Relationship between glycemic control, hyperinsulinemia and plasma concentrations of soluble adhesion molecules

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Type 2 diabetes mellitus is associated with an increased risk to develop atherosclerosis. Elevated plasma levels of circulating adhesion molecules are increased in patients with type 2 diabetes and could therefore be a potential cardiovascular risk factor. However, it is controversial whether elevated adhesion molecule plasma concentrations are primarily related to hyperglycemia or to hyperinsulinemia. We therefore evaluated the plasma concentrations of soluble E-selectin, intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) at baseline and during euglycemic hyperinsulinemic clamp in three different groups: Group A (control group): 28 healthy volunteers with normal glucose tolerance and no family history of diabetes type 2, Group B: 24 offspring of patients with type 2 diabetes with extreme insulin resistance, fasting hyperinsulinemia, normal fasting glucose but impaired glucose tolerance (IGT), Group C: 32 patients with diabetes type 2, fasting hyperinsulinemia and fasting hyperglycemia. All groups were without clinical evidence for atherosclerotic lesions, hyperlipidemia or hypertension and were matched for age, gender, and body-mass-index (BMI). Plasma soluble E-selectin, ICAM-1, and VCAM-1 levels were significantly higher ($p<0.05$) in patients with type 2 diabetes (group C) compared to the other groups. The plasma concentrations of soluble E-selectin, ICAM-1, and VCAM-1 were not higher in probands with IGT and fasting hyperinsulinemia (group B) compared to the control group (group A). The plasma soluble E-selectin, ICAM-1, and VCAM-1 levels were correlated with the fasting plasma glucose ($r=0.57$, $p<0.05$), the 2h-oGTT plasma glucose ($r=0.68$, $p<0.05$), the glycated hemoglobin ($r=0.61$, $p<0.01$), but not with the fasting insulin levels or the extent of insulin resistance, determined by euglycemic hyperinsulinemic clamp. The hyperinsulinemia during the euglycemic hyperinsulinemic clamp had no significant effect on the plasma levels of E-selectin, ICAM-1, and VCAM-1 in all three groups. In conclusion, our results suggest that elevated plasma levels of circulating adhesion molecules in patients with type 2 diabetes are rather related to hyperglycemia than to hyperinsulinemia or insulin resistance.

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DECREASED INSULIN SENSITIVITY IS ASSOCIATED WITH LOWER ANTIOXIDANT ENZYME ACTIVITY BOTH IN TYPE 2 DIABETES PATIENTS AND NONDIABETICS WITH CORONARY HEART DISEASE

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Background and Aims: It has been previously reported that decreases in insulin sensitivity (IS) are related to the impairments in systemic antioxidant enzyme activity in Type 2 diabetes patients with coronary heart diseases (CHD). Thus, the aim of this study was to compare (a) IS levels and (b) lipid peroxide levels, being important oxidative agents, and antioxidant enzyme glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities in the following groups of subjects: 15 patients with Type 2 diabetes and angiographically verified CHD (group A), 16 patients with Type 2 diabetes without CHD (group B), 14 nondiabetics with CHD (group C) and 18 healthy controls (group D). **Materials and Methods:** IS was determined by a 2h euglycemic hyperinsulinemic clamp (blood glucose targeted to 90 mg/dL, insulin infusion 1 mU/kg/min) and expressed as total glucose disposal (TGD). Lipid peroxide levels were tested in thiobarbituric acid-reacting substance (TBARS) assay and GSH-Px and SOD activity were detected by spectrophotometry. **Results:** We found that TGD was significantly lower in group A vs group B and in group C vs group D ($A: 2.3 \pm 0.8$, $B: 4.3 \pm 0.7$, $C: 4.8 \pm 1.3$, $D: 8.8 \pm 1.8$ mg/kgbw/min; A vs B and C vs D : $p<0.05$). Simultaneously, TBARS levels did not differ between groups A and group B and were slightly but not significantly higher in group C vs group D. Both GSH-Px and SOD activity were significantly lower in group A vs group B and in group C vs group D (GSH-Px: $A: 22.9 \pm 2.1$, $B: 26.2 \pm 1.9$, $C: 27.8 \pm 2.2$, $D: 32.6 \pm 2.5$ U/gHb; SOD: $A: 6.8 \pm 1.4$, $B: 9.1 \pm 1.3$, $C: 9.7 \pm 1.7$, $D: 12.1 \pm 1.6$ U/mgHb, A vs B and C vs D $p<0.05$, respectively). The changes in TGD correlated significantly both with GSH-Px and with SOD activity levels in groups A (GSH-Px: $r = 0.723$, SOD: $r = 0.704$, $p<0.05$) and C (GSH-Px: $r = 0.803$, SOD: $r = 0.787$, $p<0.05$) but not in groups B and D, while the changes in the TBARS levels did not correlate with TGD in neither of the groups. **Conclusions:** Our results signify that decreased IS is strongly associated with decreases in antioxidant enzyme activity both in Type 2 diabetes patients and in nondiabetics with CHD. The results imply that atherogenic influence of decreased IS might be mediated through an impairment of antioxidant enzyme activity both in diabetic and nondiabetic state.

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CIRCULATING E-SELECTIN, INTRACELLULAR ADHESION CELL MOLECULE-1 AND VASCULAR CELL ADHESION MOLECULE-1 IN MEN WITH CORONARY ARTERY DISEASE ASSESSED BY ANGIOGRAPHY AND DISTURBANCES OF CARBOHYDRATE METABOLISM.

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Background and aims: It is hypothesised that adhesion molecules could be an early predictor for CHD. Therefore we investigated the relationship between concentrations of soluble forms of adhesion molecules and disturbances of glucose metabolism in 78 men referred for coronary arteriography without previous history of diabetes.

Materials and Methods: 78 men (mean age 47.7 ± 6.9 years, mean BMI 27.9 ± 3.7) with symptoms of angina pectoris and positive exercise test were included in the study. In the whole group the standard OGTT with glucose and insulin estimations were performed. In the fasting plasma the concentrations of soluble forms of E-selectin, intracellular adhesion cell molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and total cholesterol, HDL-cholesterol, triglycerides, HbA_{1c} were also measured. Depending on OGTT -10.2% of the patients (n=8) fulfilled the criteria for type 2 diabetes mellitus, 44.9%-impaired glucose tolerance (n=30).

Results: The highest concentrations of E-selectin was observed in patients with type 2 diabetes mellitus and was significantly higher in comparison to the group with the normal glucose tolerance and IGT. The concentration of sVCAM-1 increased with the progression of the disturbances of the glucose metabolism and remained the highest in type 2 diabetic patients. sICAM-1 concentration was higher in men with diabetes but the difference was not statistically significant. The significant correlation between E-selectin and BMI ($r=0.41$, $p<0.0001$), fasting glycemia ($r=0.23$, $p<0.05$), post-load glycemia ($r=0.39$, $p<0.001$) and post-load insulin ($r=0.32$, $p<0.02$) was observed. Also sVCAM-1 correlated significantly with post-load insulin concentration ($r=0.27$, $p<0.05$).

Conclusions: We conclude that soluble forms of adhesion molecules and especially E-selectin could be recognised as an early marker of damaged endothelium in patients with disturbances of glucose metabolism and changes in the coronary arteries.

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Vascular Endothelial Growth Factor and ventricular remodeling in patients with type 2 diabetes and hypertension.

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Background and Aims: Recent studies have demonstrated that, in diabetic patients, Vascular Endothelial Growth Factor (VEGF) has a protective action on cardiac microcirculation but no data are available on VEGF action on cardiac morphology and function. Experimental and clinical studies have pointed out that tissue hypoxia is associated with high circulating plasma levels of VEGF. The aims of this study were: 1) to evaluate the plasma level of VEGF in hypertensive type 2 diabetic patients 2) to investigate the association between plasma VEGF levels and cardiac morphofunctional parameters. **Materials and Methods:** This study involved 29 hypertensive patients with type 2 diabetes (11 F, 18 M), mean age 61.7 ± 1.5 years, duration of disease 8 ± 7.3 years, without severe retinal or renal damage. All patients had their high blood pressure normalized to $<140/85$ mmHg BP following treatment with ACE inhibitors (n=15) and calcium antagonists (n=14). In each patient %HbA_{1c} and VEGF were evaluated together with a M-B Doppler echocardiography to determine the following morphofunctional parameters: 1) left ventricular mass (LVM), indexed LVM, relative wall thickness (RWT) to evaluate left ventricular geometry; 2) shortening fraction (SF) and ejection fraction (EF) to evaluate the systolic function; 3) transmitral early flow velocity wave/atrial early flow velocity wave (E/A) ratio and isovolumetric relaxing time (IRT) to evaluate diastolic function. **Results:** VEGF plasma levels, %HbA_{1c} and the echocardiographic parameters were not significantly different in patients treated with ACE inhibitors or Calcium antagonists. The patients were then subdivided in two groups according to their VEGF plasma levels: group A with VEGF >100 pg/ml (149.07 ± 19.10 pg/ml, n=14) and group B with VEGF <100 pg/ml (31.53 ± 22.8 pg/ml, n=15). Comparison of the groups showed that group A had a significantly bigger RWT (0.42 ± 0.06 vs 0.37 ± 0.04 , $p<0.02$). In women, plasma levels of VEGF were higher (113.27 ± 68.7 pg/ml vs 73.1 ± 56.25) and the systolic function parameters were significantly better (SF 42.36 ± 4.3 % vs 38.11 ± 7.9 %; EF 64.21 ± 4.9 % vs 58.3 ± 6.3 %, $p<0.02$) than in men. **Conclusions:** Our data demonstrate that higher levels of VEGF are correlated with a better cardiac performance and with a reduction of the abnormal ventricular remodeling, confirming the protective role of this growth factor on myocardial tissue.

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CARDIAC EXPRESSION OF NATRIURETIC SUBSTANCES IN EXPERIMENTAL DIABETES MELLITUS COMBINED WITH ANGIOTENSIN II INDUCED CARDIAC HYPERTROPHY

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Background and Aims: Cardiac expression of A- and B-type natriuretic peptides and adrenomedullin (ANP, BNP, AM) were characterized in healthy and diabetic (DM) male Wistar rats during the acute phase of angiotensin II (AII) induced cardiac hypertrophy (CH). **Materials and Methods:** DM was induced 2,5 or 7 weeks before AII treatment by a single iv. injection of Streptozotocine (60 mg/body weight kg /BW). AII (33 µg/kg/die) was administered via subcutaneously implanted osmotic mini pumps for 24 hours. Left ventricular weight to body weight ratio (LV/BW) and left ventricular expression of atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and adrenomedullin (AM) were measured (total RNA isolation by guanidine isothiocyanate-CsCl method and Northern-blotting). Four groups (n=9-13) were investigated at both time points (2,5wks and 7wks) of DM: control (C), AII treated (AII), diabetic (DM) and AII treated diabetic (AII/DM). **Results:** All groups of diabetic animals (DM and AII/DM) had significantly higher blood glucose and fructosamine values compared to controls and AII treated groups (C and AII) $p<0.05$. AII elevated the LV/BW ratio (2,5wks: 2.02 ± 0.14 (AII) vs 1.90 ± 0.09 (C) $p<0.036$, 7wks: 2.4 ± 0.12 (AII) vs 1.9 ± 0.17 (C) $p<0.048$, average \pm SD, mg/g). DM induced CH at 7wks (2.1 ± 0.11 (DM) vs 1.9 ± 0.17 (C) $p<0.0011$), but not at 2,5wks (1.96 ± 0.09 (DM) vs 1.9 ± 0.09 (C) $p<0.14$). AII treatment and DM elevated LV/BW already at 2,5 wks (2.08 ± 0.14 (AII/DM) vs 1.96 ± 0.09 (DM) $p<0.015$), without a further increase at 7wks (2.25 ± 0.17 (AII/DM) vs 2.14 ± 0.11 (DM) $p<0.129$). Left ventricular ANP expression was the best marker of CH, changes in BNP expression were similar but smaller, AM expression did not change. LV ANP expression increased 4 fold in response to AII treatment at both time points (2,5wks: 4.8 ± 0.8 (AII) vs 1 ± 0.13 (C) $p<0.001$; 7wks: 3.2 ± 0.4 (AII) vs 1 ± 0.13 (C) $p<0.0001$, average \pm SEM, densitometric values corrected for 18S protein expression, relative to control). DM alone also increased LV ANP expression (2,5wks: 3.5 ± 0.7 (DM) vs 1 ± 0.13 (C) $p<0.0025$; 7wks: 2.2 ± 0.2 (DM) vs 1 ± 0.13 (C) $p<0.0005$). AII treatment in DM further increased LV ANP expression (2,5wks 11 ± 1.4 (AII/DM) vs 3.5 ± 0.7 (DM) $p<1.4 \times 10^{-7}$; 7wks: 7.3 ± 0.9 (AII/DM) vs 2.2 ± 0.2 (DM) $p<0.00024$). **Conclusions:** The results indicate that DM induces CH, which can be further increased by acute AII treatment. ANP is the best tissue marker of CH under these experimental conditions.

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LACUNAR STROKE IN DIABETIC AND NON DIABETIC PATIENTS

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Background and Aims: In general population lacunar stroke is believed to be associated with more favourable prognosis than other ischaemic strokes. However, it is not clear whether the same phenomenon occurs in diabetic patients. The aim of this study was to assess the frequency and severity of lacunar strokes in diabetic and non diabetic patients. **Materials and Methods:** The study subjects were 109 consecutive ischemic stroke patients divided into two groups: group 1 – 41 patients with type 2 diabetes (17 men, 24 women, mean age 67.0 ± 9.5 yrs, median of diabetes duration 10.0 yrs), group 2 – 68 non-diabetic subjects (36 men, 32 women, mean age 68.0 ± 13.0 yrs). All patients had computed tomography performed on mean 6 ± 5 days of hospitalization. The subjects were subdivided according to stroke type: transient ischemic attack (TIA), lacunar infarct (LACI), total anterior circulation infarct (TACI), partial anterior circulation infarct (PACI), or posterior circulation infarct (POCI), which were diagnosed upon clinical and computed tomography examinations. The severity of stroke was assessed according to the Scandinavian Stroke Scale (SSS). The prevalence of selected risk factors such as hypertension and atrial fibrillation was evaluated. **Results:** Hypertension and atrial fibrillation were noted in 30 (73.1%) and 7 (17.1%) patients from group 1 respectively and in 49 (72.1%) and 18 (26.4%) subjects from group 2 respectively ($p>0.05$). Stroke types prevalence in diabetic and non-diabetic patients is given in the table.

	TIA	TACI	PACI	POCI	LACI
Group 1	3 (7.3)	2 (4.9)	5 (12.2)	9 (21.9)	
Group 2	11 (16.2)	5 (7.3)	19 (27.9)	11 (16.2)	22 (32.3)

Data are n (%); * $p<0.01$ vs group 2.

In diabetes group there was no statistical significant difference between neurological deficit associated with LACI vs other ischemic strokes (SSS score 36.5 and 33.2 points; $p>0.05$), while in non-diabetic group LACI was associated with milder neurological impairment when compared with other ischemic stroke types (SSS score 38.3 and 32.4 ; $p=0.036$). **Conclusions:** The obtained results suggest that lacunar stroke is more frequent and leads to more significant neurological impairment in diabetic than non-diabetic subjects.

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RESISTANCE TO ACUTE INSULIN INDUCED DECREASES IN LARGE ARTERY STIFFNESS IS A COMPONENT OF THE INSULIN RESISTANCE SYNDROME

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Background: Arterial stiffness has recently been recognized as an important cardiovascular risk marker. Physiological concentrations of insulin diminish wave reflection in the aorta *in vivo*. This decreases central blood pressure augmentation and augmentation divided by pulse pressure (the augmentation index, AgI), a measure of arterial stiffness. The aim of the present study was to examine whether a defect in this action of insulin is a feature of insulin resistance, and how it relates to other acute actions of insulin including stimulation of glucose uptake, peripheral blood flow and autonomic control of heart rate variation. **Materials and methods:** Actions of insulin were quantitated in 50 healthy men (age 34±2 yrs, body mass index 27±1 kg/m²) during 2 sequential insulin infusions each lasting 120 min (1 and 2 mU/kg·min). Insulin action on arterial stiffness (AgI) was measured with pulse wave analysis, on peripheral blood flow with venous occlusion plethysmography and on autonomic control of heart rate variation with spectral power analysis. **Results:** Insulin decreased AgI significantly within 30 min, while significant increases in peripheral blood flow and normalized low frequency power of heart rate variation (LFn), a measure of sympathetic control of heart rate variation, were observed at 150 min and 210 min. A blunted decrease in the AgI was significantly associated with a low rate of insulin stimulated glucose uptake, but not with the other actions of insulin. Insulin action of the AgI was correlated with body mass index and the waist to hip ratio independent of basal AgI, age and LDL cholesterol. **Conclusions:** Physiological concentrations of insulin diminish large artery stiffness within 30 min in non-diabetic men. This action precedes insulin action on peripheral vasodilatation, heart rate and autonomic control of heart rate variation. It is correlated with insulin stimulation of glucose uptake and is blunted by known causes of insulin resistance including overall and abdominal obesity. Resistance of large arteries to insulin induced decrease in their stiffness is therefore another facet of insulin resistance which could contribute to the association between insulin resistance and cardiovascular disease.

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CAROTID ATHEROSCLEROSIS IN DIABETES MELLITUS AND IN ISCHEMIC HEART DISEASE: ULTRASONOGRAPHIC EVALUATION

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Background and Aims: Diabetic patients without ischemic heart disease (IHD) have a risk of myocardial infarction (MI) similar to non-diabetic subjects with previous MI. Since carotid atherosclerosis (CA) is an important marker of coronary atherosclerosis, we used carotid ultrasonography to evaluate if diabetic patients without IHD show a CA level similar to non-diabetic subjects with IHD. **Materials and Methods:** Using a high-resolution B-mode ultrasound (Hewlett Packard Sonos 5500) equipped with a 7.5 MHz imaging transducer, we studied 782 subjects (41.2 % male), ageing ≥ 55 years (69.8 ± 8.9 years), 598 without cardiovascular disease (CVD) nor diabetes (DM) (N), 74 diabetics without CVD (D), 74 non-diabetics with IHD (I) and 36 diabetics with IHD (D+I), consecutively attending our vascular ambulatory. Three longitudinal views of the common, bifurcation and internal carotid arteries were examined bilaterally, searching for the maximum intima-media thickness (IMT). CA was classified as: absent (ABS) (IMT < 0.9 mm); thickened (THI) (IMT ≥ 0.9 and < 1.5 mm); plaque (PL) (IMT ≥ 1.5 mm and internal carotid systolic velocity [CSV] < 125 cm/s); stenosis (ST) (IMT ≥ 1.5 mm and internal CSV ≥ 125 cm/s). **Results:** The percentage of patients in the combined PL and ST classes were 67.7%, 83.8%, 79.7%, and 91.7% for N, D, I and D+I, respectively (p < 0.05-0.001 N vs D, I, and D+I). Compared to N, the prevalence of D (ABS: 4.1%, THI: 6.4%, PL: 13%, ST: 14.8%, p = 0.0057), I (ABS: 6%, THI: 7.6%, PL: 10.2%, ST: 23.3%, p = 0.0007) and D+I (ABS: 0%, THI: 2%, PL: 5.6%, ST: 15.9%, p < 0.0001) increased significantly in the more advanced CA classes. After adjustment for age, sex and the common cardiovascular risk factors, the progression of CA classes was significantly associated with D (Odds Ratio [OR] for trend 1.67 [C.I. 1.28-2.07], p = 0.01), as well as with I (OR 1.50 [C.I. 1.13-1.88], p = 0.03). The OR for the association of CA classes with D+I showed an additive value (OR 3.74 [C.I. 3.11-4.37], p < 0.001). **Conclusions:** Diabetic patients without IHD show a CA severity similar to non-diabetic subjects with IHD. The coexistence of DM and IHD exhibits an additive effect on the CA severity.

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INTIMA-MEDIA THICKNESS IS ASSOCIATED WITH GLUCOSE STIMULATED INSULIN SECRETION IN FIRST-DEGREE RELATIVES OF TYPE 2 DIABETIC PATIENTS AND THEIR CONTROLS

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Background and Aims: Endothelial dysfunction is associated with insulin resistance in normoglycemic first-degree relatives (FDR) of type 2 diabetic patients. In this study, we examined whether beta-cell function and/or insulin sensitivity is associated with intima-media thickness (IMT) of the right carotid artery in FDR and healthy control subjects.

Materials and Methods: We recruited 45 normotensive and normoglycemic male FDR and 40 male healthy non-diabetic control subjects for the study. They were investigated with an intravenous glucose tolerance test followed by an insulin injection iv (Minimal Model) and ultrasound measurement of the right carotid bulb.

Results: Age (44±1 vs 45±1 yrs (Mean±SE), BMI (25.7±0.4 vs 24.6±0.5 kg/m²), acute insulin response (AIR) (0-10 min) following 0.3g/kg glucose iv (409±45 vs 381±59 mU/l) and IMT (0.797±0.033 vs 0.755±0.033 mm) was similar in the groups. However, HbA1c was higher (4.7±0.06 vs 4.3±0.04 %, p<0.0001) and insulin sensitivity (IS) decreased (330±36 vs 420±41 μU/mU/min, p<0.05) in FDR. Furthermore, there was an association between IMT and AIR (rs:0.274, p=0.0148), IMT and WHR (rs:0.248, p=0.025), but not for IMT and IS (rs:-0.094, p=0.404) in the groups (n=85).

Conclusions: IMT may be more strongly associated with beta-cell function than with peripheral insulin sensitivity in FDR and healthy control subjects.

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SERUM LEVELS OF NITRIC OXIDE METABOLITES AND INDUCIBLE NO-SYNTASE IN OBESE AND NON-OBESE DIABETIC SUBJECTS

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Background and Aims: The disturbances of nitric oxide (NO) production and activity have been implicated in the pathogenesis of diabetes and its complications. However, the association between changes in NO production and obesity in diabetic patients remains not fully understood. Therefore, the aim of the study was to investigate serum levels of NO metabolites - NO₂ and NO₃ and inducible NO-synthase (iNOS) in obese and non-obese diabetic patients.

Materials and Methods: We studied 10 diabetic patients with obesity (age: 56.8±2.2 years, BMI: 35.3±0.8 years, waist/hip ratio (WHR) - 0.98±0.03), 9 patients with diabetes without obesity (age: 52.1±1.6 years, BMI: 24.2±0.8 kg/m²; WHR - 0.88±0.02), and 19 non-diabetic subjects - 9 obese (age: 52.8±2.1 years, BMI: 33.9±0.6 kg/m²; WHR - 0.89±0.02), and 10 non-obese (age: 47.4±1.4 years, BMI: 24.0±0.6 kg/m²; WHR - 0.85±0.02). Serum NO₂ and NO₃ levels were determined by spectrophotometry. Statistical analysis was performed by Student's paired test.

Results: There were no significant changes of the levels of NO₂ and NO₃, iNOS between obese and non-obese subjects either in the group of diabetic or non-diabetic persons. However, we found an increase of NO₂, NO₃, iNOS serum levels in patients with diabetes compared to control subjects in obese and non-obese ones. In obese subjects NO₂ serum levels were 0.10±0.02 and 0.03±0.01 nmol/mg of protein, p<0.05, NO₃ - 7.8±0.8 and 4.7±1.1 nmol/mg of protein, p<0.05, iNOS - 23.2±3.3 and 11.2±1.7 nmol/mg of protein, p<0.05, in those with and without diabetes, respectively. In non-obese subjects this trend was less pronounced - NO₂ levels were 0.07±0.02 and 0.04±0.01 nmol/mg of protein, p=0.06, NO₃ - 8.6±2.2 and 4.6±1.2 nmol/mg of protein, p=0.07, iNOS - 18.4±3.0 and 12.9±1.6 nmol/mg of protein, p=0.07, in those with and without diabetes, respectively.

Conclusions: The revealed changes of NO₂, NO₃, iNOS serum levels could reflect an activation of NO pathway in patients with diabetes while the difference in body mass does not seem to contribute to this activation.

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HYPERGLYCEMIA INCREASES NITRIC OXIDE PRODUCTION IN CARDIAC FIBROBLASTS FROM HYPERTENSIVE RATS

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Background and Aims: Clinical results indicate that there is higher mortality after myocardial infarction in diabetic patients with hypertension. In the ischemic myocardium, an increased synthesis of interleukin-1β (IL-1β) has been observed. This cytokine stimulates nitric oxide (NO) production by regulating inducible NO synthase (iNOS) expression. Several laboratories have also showed that cardiac fibroblasts (CF) are a main source of NO in the post infarction period. Therefore, the aim of our study was to investigate the effect of hyperglycemic conditions on IL-1β-induced NO production and iNOS expression in these cells from hypertensive and normotensive rats. **Materials and Methods:** Cultured adult CF from normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats were used. Cells were incubated with either 5 mM (control) or 22 mM (hyperglycemic conditions) glucose for 72 hours, with or without IL-1β (0.1 ng/ml) in the last 24 hours. Nitrite production by Griess reaction and iNOS expression by Western-blotting were determined. **Results:** High glucose modestly increased NO production in basal conditions in CF from both strains (WKY: 12.70±1.72 nmol/mg protein (control), 24.22±3.83 nmol/mg protein (hyperglycemia) p<0.02; SHR: 14.77±1.12 nmol/mg protein (control), 28.86±2.19 nmol/mg protein (hyperglycemia) p<0.001). This effect was significantly enhanced when the cells were stimulated with IL-1β, and was higher in the cells from hypertensive strain (WKY: 38.06±8.43 nmol/mg protein (control), 98.05±16 nmol/mg protein (hyperglycemia) p<0.01, SHR: 72.65±3.83 nmol/mg protein (control), 139.60±7.58 nmol/mg protein (hyperglycemia) p<0.001). In accordance to this, CF from both strains stimulated with IL-1β showed an enhanced iNOS expression under hyperglycemic conditions. This increase on NO production and iNOS expression was not modified by inhibition of protein kinase C (calphostine 0.2 μM) or superoxide dismutase (300 U/ml) but reversed with tunicamycin (0.5 μg/ml), an inhibitor of N-linked glycosylation. **Conclusions:** These results indicate that hyperglycemia increases NO production and iNOS expression in CF stimulated with IL-1β, this effect being higher in cells from SHR. Hyperglycemia effect does not seem to be related to PKC activation or an increased superoxide anion production but mediated by N-linked glycosylation of proteins. The higher increase on NO production observed in CF from hypertensive animals might be in accordance to the worse prognosis that after myocardial infarction show the diabetic patients with hypertension. [Supported by DGICYT BXX2000-0153 and Lilly].

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EFFECTS OF AMINOGLUCANIDINE ON REACTIVE OXYGEN SPECIES AND NITRIC OXIDE RELEASE IN TARGET TISSUES OF DIABETIC RATS

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Background and Aims: Increased oxidative stress is associated with tissue damage in diabetes. Glycooxidation is one of the sources of permanent oxidative damage in diabetic animals and humans. The aim of this study was to investigate the effects of aminoguanidine (AG), which is an inhibitor of advanced glycation endproducts and nitric oxide (NO) synthesis, on reactive oxygen species (ROS) and NO release from the target tissues of diabetic rats. **Materials and Methods:** Diabetes was induced by streptozotocin in 10 male wistar rats. After 1 week, 6 of the diabetic rats (Group A) were given AG hydrogen carbonate 1 gr/L in drinking water ad libitum; 4 diabetic rats were given tap water (Group B). Six healthy rats were followed as control (Group C). At the end of 6 week period skin, kidney and heart tissue samples were collected and the following parameters were measured. Glucose determined with glucosidase. NO release was established with luminol and H₂O₂ system. ROS were measured by luminol and lucigenin enhancement with chemiluminescence. **Results:** Plasma glucose levels were 92.6±7.5, 260.7±84, 251.6±53.4 mg/dl for group C, B, A respectively. Diabetic groups have higher levels than healthy controls (p<0.001). NO measurements were 281.7±45.2, 610.7±267, 231±93 fmol.min⁻¹(g tissue weight)⁻¹ in skin; 124.3±52.3, 329.3±115, 129.6±23.5 fmol.min⁻¹(g tissue weight)⁻¹ in kidney; 286.8±88.7, 411.7±141, 285.9±122 fmol.min⁻¹(g tissue weight)⁻¹ in heart tissues for group C, B, A respectively. Group B had higher level of NO release than other groups (p<0.05). Luminol enhanced measurements were 151.7±20.9, 217.4±71.8, 141.1±37 AUC rlu/mg in skin; 105.9±30.2, 190.2±57.2, 118.3±36.1 AUC rlu/mg in kidney; 125.6±21.2, 169.3±74.2, 132.4±54 AUC rlu/mg in heart for group C, B, A respectively. Lucigenin measurements were 195.2±43.5, 262.2±85.2, 130.9±30.3 AUC rlu/mg in skin; 154.6±18.6, 256.8±90.7, 134.4±9.3 AUC rlu/mg in kidney; 139.2±43.1, 182.7±90, 170.5±39 AUC rlu/mg in heart for group C, B, A respectively. Skin and kidney tissues of diabetic controls had higher levels of both luminol (p<0.05) and lucigenin (p<0.01) enhanced ROS measurements compared to group A and C. NO release was found to be correlated with luminol (r=0.62, p<0.0001) and lucigenin (r=0.51, p<0.002) measurements in all tissue samples of 3 groups. **Conclusion:** ROS and NO release were significantly increased in skin and kidney tissues of diabetic rats. Increased peroxynitrite formation seems to play a major role in tissue damage in short term period in our model. AG treatment lowered NO release and peroxynitrite formation. This could be one of the mechanisms by which AG protects

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PLASMA HOMOCYSTEINE AND CHRONIC COMPLICATIONS IN TYPE 1 DIABETES.

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Background and Aims: Elevation of plasma homocysteine (Hcy) is independently associated in type 2 diabetes with a higher prevalence of nephropathy and macroangiopathy. In type 1 diabetes, only a handful of rather contradictory studies have dealt with this relationship. Therefore, we determined the distribution of Hcy concentrations in a selected cohort of type 1 diabetic patients and assessed whether Hcy values were independently related to chronic complications. **Materials and Methods:** 71 consecutive patients (48% males) admitted to the ward for poor glycaemic control and/or treatment of complications, especially macroangiopathy, were included. Age and duration of diabetes (DD) were 51 (34-63) and 23 (13-32) years (median [percentile 25-75]), respectively. Body mass index (BMI) was 24 (21-26) kg/m² while systolic and diastolic blood pressure (BP) levels were 130 (120-150) and 80 (70-80) mmHg. 14 and 28% were past or current smokers. Antihypertensive and/or hypolipidemic drugs and/or aspirin were given to 13, 42 and 19% of subjects. HbA_{1c} was 9.3 (8.2-10.5)%. Neuropathy, retinopathy, nephropathy and macroangiopathy were present respectively in 60, 62, 30 and 41% of patients. Plasma Hcy was determined in the fasting state using a FPIA-IMx analyser (Abbott). **Results:** Hcy was 9.2 (4.7-26.2) μmol/L. Raised Hcy levels (> 15.0 μmol/L) were present in 15% of the cohort. Vitamin B₁₂ and folic acid were 561 (426-698) μg/ml and 5.7 (4.4-7.3) ng/ml. Univariate statistical analysis showed a positive association between Hcy and age (P<0.001), DD (P<0.001), systolic BP (P<0.001), plasma creatinine (P<0.001), cholesterol (C)/HDL-C (P=0.021), neuropathy (P<0.001), retinopathy (P<0.001) and nephropathy (P<0.001). Hcy was also higher in patients with than without macroangiopathy (13.2 [9.6-15.7] vs. 7.8 [6.5-9.2] μmol/L, P<0.001). No association was observed with HbA_{1c}. A negative correlation was found with folic acid (P=0.014) and creatinine clearance (P<0.001). When major variables (sex, age, diabetes duration, BMI, BP, smoking, HbA_{1c}, lipids) were taken into account for multiple logistic regression, we confirmed an independent association of Hcy with age (P=0.003), plasma creatinine (P<0.001) and folic acid (P=0.014), but not with vascular complications. **Conclusions:** Increased Hcy is present in 15% of poorly-controlled type 1 diabetic patients and is associated with age, creatinine and folic acid levels. In this type 1 population, there was no independent correlation of Hcy with macroangiopathy.

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IMPROVED GLYCEMIC CONTROL IN TYPE 2 DIABETES PATIENTS IS ASSOCIATED WITH DECREASE IN TOTAL PLASMA HOMOCYSTEINE
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Background and Aims: It has been shown that homocysteine, a newly established risk factor for cardiovascular disease, tends to be increased in type 2 diabetes mellitus. Animal studies have suggested that insulin may play a role in homocysteine metabolism regulation. The study aimed at assessing the effect of improved glycemic control achieved with insulin treatment on total plasma homocysteine (tHcy) in poorly controlled type 2 diabetes patients. **Materials and Methods:** This 6-month prospective study comprised 78 subjects divided into 3 groups: group 1 – 30 type 2 diabetes patients (mean age 62.6±7.9 yrs) in secondary failure to sulfonylureas, in whom insulin therapy was initiated at baseline; group 2 – 30 type 2 diabetes patients (mean age 61.2±8.4 yrs) sufficiently treated with sulfonylureas and maintaining their treatment throughout the study; group 3 – 18 healthy subjects (mean age 63.0±6.3 yrs) serving as controls. Fasting tHcy, folic acid, vitamin B₁₂, and HbA_{1c} levels were assessed at baseline and after 6-month period. tHcy was measured with high performance liquid chromatography (HPLC), folic acid and vitamin B₁₂ – microparticle immunoenzymatic method MEIA, HbA_{1c} – low pressure chromatography. **Results:** Mean (±SD) total plasma homocysteine and glycated hemoglobin HbA_{1c} are shown in the table.

group	HbA _{1c} (%)		tHcy (μmol/l)	
	baseline	after 6 months	baseline	after 6 months
1	10.8±1.6 ^{a,b}	8.1±1.5 ^c	14.4±2.4 ^{a,b}	11.0±3.6 ^c
2	6.9±0.4 ^d	7.2±0.5 ^d	8.4±3.1	8.1±2.8
3	5.1±0.3	5.0±0.3	6.9±2.7	7.2±2.9

^ap<0.001 vs after 6 months' values in group 1; ^bp<0.0001 vs all values in group 2 and 3; ^cp<0.05 vs all values in group 2 and 3; ^dp<0.01 vs all values in group 3. Folic acid and vitamin B₁₂ concentrations were within their reference ranges at baseline and after 6 months' follow-up in all study subjects. **Conclusions:** Improved glycemic control achieved with insulin therapy in type 2 patients was associated with decrease in total plasma homocysteine level.

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Abnormal neutrophil actin assembly is associated with increased Beta2integrin expression in Type 2 diabetes.

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Background and Aims: Neutrophil dysfunction is common in patients with diabetes. Many neutrophil functions rely on remodeling of the actin cytoskeleton. Patients with Type 2 diabetes are also at increased risk of cardiovascular disease. Neutrophils are implicated in the pathogenesis of cardiovascular disease through their expression of the beta2integrin, Mac-1, which is a dimer of CD11b and CD18. We examined neutrophil actin assembly in Type 2 diabetes and its effect on CD11b expression in response to activation with PMA. **Materials and Methods:** Type 2 diabetic patients (T2DM) were from the regional diabetes centre- fasting blood glucose >7.0 mmol/l or 2 hour post glucose load level >11.1mmol/l (n=11, mean age 58.5 years). Age of diagnosis of Type 2 diabetes was >40 years, arterial pressure <140/90mmHg; BMI <30Kg/m² and there was absence of microalbuminuria on 3 separate albumin:creatinine ratios. Normal controls (NC) had a fasting blood glucose <7.0mmol/L (n=15, mean age 62.7 years). Blood cells were incubated with 100ng/ml PMA for 30 minutes, stained with anti-CD11b-PE, fixed, permeabilised and stained with FITC-phalloidin, which binds F-actin. Samples were analysed by Faccscanner. Results are means ± S.E. and analyses by Student's T-test.

Results: In both NC and T2DM there were three populations of neutrophil- (1)Low phalloidin-binding: NC- 50.7±2.6% cells, 13326±1389 phalloidin-binding sites, 56234±6307 CD11b sites; T2DM- 59.2±2.9% cells, 15571±2952 phalloidin-binding sites, 45101±5035 CD11b sites. (2)Medium phalloidin-binding: NC- 11.6±1.4% cells, 94996±12307 phalloidin-binding sites, 46756±7714 CD11b sites; T2DM- 11.5±1.5% cells, 123228±14058 phalloidin-binding sites, 39675±4985 CD11b sites. (3)High phalloidin-binding: NC- 37.7±1.9% cells, 222717±15523 phalloidin-binding sites, 208±169 CD11b sites; T2DM- 29.2±2.1% cells, 237695±16593 phalloidin-binding sites, 405±180 CD11b sites.

The proportion of cells with high levels of phalloidin-binding and hence increased F-actin was significantly higher in NC than T2DM (p=0.0023). In both groups, cells with high levels of phalloidin-binding had significantly less CD11b sites (p<0.0001). In T2DM there was not an associated increase in cells with medium-binding suggesting a defect in the initiation of actin assembly in response to PMA.

Conclusions: Neutrophil actin assembly is impaired in Type 2 diabetes. This is associated with an increase in the expression of CD11b which is important in the pathogenesis of cardiovascular disease through increased neutrophil-endothelial binding.

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DYSFUNCTION OF POLYMORPHONUCLEAR NEUTROPHILS IN PATIENTS WITH TYPE I DIABETES

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Background and aims: Polymorphonuclear neutrophils (PMNs) play an important role in the pathogenesis of diabetic vascular complications. Stimulation of these cells is associated with the appearance of specific receptors on their surface and desquamation of L-selectin. The aim of the study was to evaluate the serum concentration of L-selectin and the expression of specific PMN receptors: CD11b, CD18. **Materials and methods:** the study was performed in a group of 80 Type I diabetic patients, aged 30.7 ± 9.7 years, 46 females and 34 males, with diabetes duration 13.0 ± 8.0 years; HbA_{1c} 7.80 ± 2.34 %; fructosamine 348.6 ± 99.4 μmol/l; FPG 8.3 ± 2.8 mmol/l. The serum concentration of L-selectin was estimated with the use of the ELISA test. The expression of PMN surface receptors was measured by flow cytometry using Ortho-diagnostic system cytofluorimeter. The results were presented as a PMN percentage indicating expression of CD11b and CD18 and as mean channel fluorescence intensity (MFI). **Results:** in comparison with healthy subjects we observed significantly higher serum concentration of L-selectin in Type I diabetic patients (954.0 ± 13.0 vs 1373.9 ± 232.1 ng/l, p<0.05). The percentage of PMN with presence of CD11b and CD18 receptors was significantly higher (CD11b: 98.7 ± 0.6 vs 93.2 ± 3.4 %, p<0.05; CD18: 99.5 ± 0.5 vs 98.1 ± 0.5%, p<0.05) but the MFI values for CD11b and CD18 were significantly decreased in diabetic patients in comparison with controls (MFI CD11b: 148.0 ± 9.9 vs 159.0 ± 5.6, p<0.01 and MFI CD18: 144.2 ± 9.5 vs 161.7 ± 3.0, p<0.01). We did not find any correlation between expression of CD11b/CD18, serum concentration of L-selectin and duration of the disease, HbA_{1c}, fructosamine, FPG or presence of late diabetic complications. **Conclusions:** the results suggest enhanced activity of unstimulated PMNs and may indicate their lower response after stimulation.

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INSULIN ABILITY TO STIMULATE PAI-1 RELEASE IS PRESERVED IN FIBROBLASTS FROM INSULIN RESISTANT INDIVIDUALS.

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Background and Aims: Plasma PAI-1 levels are increased in insulin resistant states and impaired fibrinolysis might be one of the links between insulin resistance and accelerated atherosclerosis. Insulin can stimulate PAI-1 synthesis and gene expression in a variety of cell types, including hepatocyte, vascular smooth muscle cells and fibroblasts. However, in order for the insulin resistance induced hyperinsulinemia to be able to increase PAI-1 in vivo, sensitivity to insulin stimulation of PAI-1 synthesis needs to be preserved in the presence of insulin resistance in glucose metabolism. To test whether this was true, insulin effect on PAI-1 release in the culture medium was studied in fibroblasts from individuals with different degrees of insulin resistance.

Material and Methods: Six fibroblast strains were cultured from skin biopsies obtained from 3 insulin sensitive (clamp M > 7 μmol/Kg/min) and 3 insulin resistant (clamp M < 5 μmol/Lg/min) volunteers matched for age and BMI. On each strain, insulin stimulation (10nM) of 14C 2-deoxy-glucose uptake, glycogen synthesis and PAI-1 release in the culture medium were measured on separate experiments.

Results: In fibroblasts from insulin resistant individuals, insulin stimulated glucose uptake and glycogen synthesis less as compared to insulin sensitive individuals (125±8.5 vs 188±39.6 μmol/g prot, p<0.05 and 0.58±0.02 vs 0.74±0.03 mg glycogen/g protein, p<0.01, respectively). However, insulin stimulated PAI-1 release in the culture medium was not different in fibroblasts from insulin sensitive and insulin resistant individuals (41.9±7.8 vs 35.9±12.7 ng/ml, p=n.s.).

Conclusions: This data show that insulin ability to stimulate PAI-1 release is preserved in cells from insulin resistant individuals in which glucose uptake is resistant to insulin stimulation. Thus, in the insulin resistance syndrome, hyperinsulinemia might be one of the culprit for the observed increase in PAI-1 levels.

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Cessation smoking decrease transforming growth factor β_1 plasma and urinary levels in patients with type 1 diabetes mellitus.
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Background and Aims. Tobacco consumption has been implicated in increased risk for diabetic nephropathy. Transforming growth factor β_1 (TGF β_1) plays a role in the pathogenesis of this complications. The aim of our study was to prospectively assess the effects of smoking cessation on TGF β_1 plasma and urinary levels in cigarette smokers with type 1 diabetes mellitus (DM). **Patients and Methods.** Sixteen patients (9 females, 7 males; aged 30 ± 4 years) with type 1 DM (mean duration 12 ± 7 years), BMI 25 ± 2 kg/m², with normal urinary albumin excretion (UAE), who smoked > 15 cigarettes/day, were included in a smoking cessation program. Before and at 1 week, 4 weeks and 12 weeks after cessation of smoking plasma and 24-h urinary TGF β_1 levels were determined. Urinary cotinine was measured as an index of smoking consumption and abstinence. The total plasma and urinary concentrations of TGF β_1 were measured by enzyme-linked immunoassay. **Results.** TGF β_1 and UAE were logarithmically transformed before statistical analysis. The main results are shown in the following table:

	0	Weeks		
		1	4	12
n	16	16	11	9
TGF- β_1 (ng/ml)	12.4 \pm 7.3	9.1 \pm 6.9	8.8 \pm 7.1	5.8 \pm 4.0*
TGF- β_1 (ng/g creatinine)	16.9 \pm 8.7	14.2 \pm 8.8	14.2 \pm 8.7	10.3 \pm 2.0*
Cotinine (ng/ml)	1473 \pm 528	16.5 \pm 4.2	0	0
Weight (Kg)	71.0 \pm 11.8	71.3 \pm 11.0	72.2 \pm 10.0	72.5 \pm 10.3

*p 0.01 vs. week 0

Conclusions. Our data suggest that cessation smoking in diabetic patients produces a reduction in TGF β_1 plasma and urinary levels. this is an additional argument to encourage diabetic patients to give up smoking.

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Plasma and urinary transforming growth factor β_1 levels in patients with type 1 diabetes mellitus and microalbuminuria
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Background and Aims. Transforming growth factor β_1 (TGF β_1) has been implicated in the pathogenesis of diabetic nephropathy (DN). The aim of our study was to evaluate TGF β_1 activity in type 1 diabetes mellitus (DM) with and without microalbuminuria and non-diabetic controls. **Patients and Methods.** We evaluated TGF β_1 plasma and 24-h urine levels from 20 non-diabetic subjects and 50 patients with type 1 (DM), 15 with microalbuminuria and 35 with normal urinary albumin excretion (UAE). All patients were normotensive and had not been taking angiotensin II (Ang II) converting enzyme inhibitor or Ang II antagonist drugs. Total plasma and urinary concentration of TGF β_1 were measured by enzyme-linked immunoassay. **Results.** TGF β_1 and UAE were logarithmically transformed before statistical analysis. The main results are shown in the following table.

	controls	DIABETES	
		normoalbuminuric	microalbuminuric
n:	20	35	15
Age (yr)	34.2 \pm 7.9	33.0 \pm 8.1	31.8 \pm 10.1
Diabetes duration (yr)	-	13.0 \pm 5.1	17.2 \pm 7.9
TGF- β_1 (ng/ml)	4.7 \pm 3.7*	10.8 \pm 8.6	11.4 \pm 7.7
TGF- β_1 (ng/g creatinine)	5.3 \pm 2.2*	12.1 \pm 5.1	12.2 \pm 7.8

* P 0.01, controls vs. diabetic patients; yr: years

In normoalbuminuric group, the smoker patients had plasma (15.5 \pm 10.0 ng/ml) and urinary TGF β_1 levels (14.3 \pm 3.7 pg/g creatinine) higher than non-smokers (7.9 \pm 6.2 ng/ml and 10.9 \pm 5.5 pg/g creatinine in plasma and urine, respectively. p < 0.04). **Conclusions:** We concluded that TGF β_1 activity is higher in Type 1 DM patients than in control subjects. However, these parameters not difference between normoalbuminuric and microalbuminuric patients. Increased TGF β_1 activity in smokers is consistent with the hypothesis that smoking is an important factor in the development DN, providing a mechanistic link between smoking and DN.

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Evidence for a novel transforming growth factor- β_1 -independent mechanism of fibrosis in mesangial cells overexpressing glucose transporters
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Background and Aims: Recent experimental work indicates that the hyperglycemia-induced increase in mesangial matrix production, a hallmark in the development of diabetic nephropathy, is mediated by an increased expression of glucose transporter 1 (GLUT1). Mesangial cells stably transfected with the human GLUT1 (GT1 cells) mimic the effect of hyperglycemia on the production of extracellular matrix proteins when cultured under normoglycemic conditions. In the present study we investigated the molecular mechanism of the increased matrix production in GT1 cells.

Materials and Methods: GT1 cells and LacZ cells overexpressing β -galactosidase as control cells were cultured in experimental medium containing 6 mM glucose. Supernatants (w/o foetal calf serum) were collected for determination of fibronectin and transforming growth factor- β_1 (TGF- β_1) by ELISA. Total RNA was prepared for northern blotting, cellular or nuclear extracts were prepared for western blotting or electrophoretic mobility shift assays. Hexosamines were measured by capillary electrophoresis and generation of reactive oxygen species by FACS.

Results: While in normal mesangial cells the hyperglycemia-induced matrix protein expression is mediated through overexpression of TGF- β_1 we found no increased expression of TGF- β_1 in the GT1 cells and addition of anti-TGF- β_1 antibodies did not prevent the enhanced fibronectin production. Because fibronectin and other matrix proteins contain AP-1 responsive elements in their promoters the activation of this transcription factor was studied in GT1 cells. We found strong increases in the nuclear amount of Jun proteins leading to enhanced DNA binding activity of an AP-1 complex. Addition of the AP-1 inhibitor curcumin significantly reduced the level of secreted fibronectin protein. In contrast to mesangial cells exposed to high glucose no activation of the hexosamine biosynthetic pathway, the p38 or the ERK1/2 MAPK pathway could be detected, which can be explained by the absence of oxidative stress in the GT1 cells.

Conclusions: Our data indicate that in mesangial cells increased glucose uptake under normoglycemic conditions induces fibronectin production via activation of pathways different from hyperglycemia-induced activation. Therefore, TGF- β_1 -dependent and/or -independent pathways may be involved in the development of diabetic nephropathy.

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THE ROLE OF SRC HOMOLGY 2 CONTAINING PROTEIN TYROSINE PHOSPHATASE 2 (SHP2) ON PROLIFERATION OF RAT SMOOTH MUSCLE CELL

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Background and aims Src homology-containing protein tyrosine phosphatase 2 (SHP2) is ubiquitously expressed and believed to function as part of the positive signaling pathway mediating growth factor-induced protein tyrosine phosphorylation. The increased proliferation in aortic vascular smooth muscle cells (SMC) is one of the important causes in atherosclerosis. The aim of this study was to examine the proliferative effect of SHP2 expressed in SMC.

Method and results SHP2 was abundantly expressed in SMC and stained in thickening intima in the balloon-injured rats. We obtained several cloned SMC using geneticin. SHP2 in each clone has various ranges of endogenous expressions. There is significantly positive correlation between SHP2 expression and BrdU uptake stimulated by FBS, platelet derived growth factor or insulin-like growth factor (p<0.05). In transiently SHP2 transfected SMC, FBS stimulation significantly increased BrdU uptake compared to control SMC (p<0.05).

Conclusion These data suggest that an increased expression of SHP2 may lead to the acceleration of atherosclerosis in aorta through the action of increasing cell growth.

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HUMAN G-PROTEIN $\beta 3$ -SUBUNIT C825T GENE POLYMORPHISM AND EARLY ATHEROSCLEROSIS IN TYPE 1 DIABETIC PATIENTS

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Background and Aims: Human G-proteins play a major part in transmembrane signal transduction. A cytosine to thymidine exchange in codon 825 of the gene coding for the $\beta 3$ subunit of the G-Protein (GNB3) leads to the synthesis of a splice variant, which results in a significantly enhanced activation of the G-protein due to the increased proliferation of smooth muscle cells. This in turn leads to vascular hypertrophy. The T-allele has recently been associated with arterial hypertension. The aim of this study was to evaluate the role of this GNB3 C825T gene polymorphism for early atherosclerosis in type 1 diabetic patients.

Materials and Methods: We determined the C825T genotype by PCR and subsequent restriction enzyme analysis with Bse D1 in 146 type 1 diabetic patients (91 women, 55 men; age 30.1 \pm 6.6 years, diabetes duration 13.0 \pm 8.1 years). The carotid artery intima-media thickness (IMT), which reflects early atherosclerosis, was measured by high resolution ultrasound.

Results: The genotype distribution was 48 % CC, 43 % TC and 9% TT. There were no significant differences in age, diabetes duration or metabolic control between patients with different genotypes. The IMT was 0.61 \pm 0.11 mm in the CC, 0.65 \pm 0.18 mm in the TC and 0.56 \pm 0.11 mm in the TT genotype (n.s.).

Conclusions: No correlation of the GNB3 C825T polymorphism with IMT was observed. These findings do not support the hypothesis that the GNB3 C825T gene polymorphism is a risk factor for early atherosclerosis in young type 1 diabetic patients.

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ASSOCIATION OF THE TNF- α GENE POLYMORPHISMS WITH CORONARY ARTERY DISEASE IN TYPE 2 DIABETES MELLITUS

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Background and Aims: Proinflammatory cytokines such as Tumor Necrosis Factor (TNF- α) and the presence of infections in the vessel wall mediated by lipopolysaccharides have been implicated in the pathogenesis of coronary heart disease. We hypothesized that polymorphisms of the TNF- α and lipopolysaccharide receptor (CD14) genes may be associated with a predisposition to coronary heart disease (CHD) in type 2 diabetes.

Materials and Methods: 341 CHD patients, 207 matched control subjects and 135 type 2 diabetic patients without CHD were evaluated. Two SNPs at the promoter TNF- α (-308 and -863) and one at the -159 CD14 gene associated with variations in the transcription rate of these genes were analyzed by RFLP-PCR.

Results: Genotype frequency for TNF- α /-308 T2 allele was significantly greater in the CHD group than in controls (32.3% vs 23.2% respectively; $p=0.03$). The remaining genotype frequencies were similar in cases and control subjects. Among CHD patients, the TNF- α -308 T2 allele was preferentially associated in those having type 2 diabetes mellitus (28.5% vs 40.6% respectively; $p=0.0056$). When we compare the allelic frequencies in type 2 diabetic subjects, T2 allele was carried in 40.6% of diabetic CHD patients compared to 19.3% in diabetic non-CHD patients ($p=0.00048$; OR=1.55-5.32). A logistic regression analysis demonstrated that TNF- α -308 T2 allele was an independent determinant of CHD in type 2 diabetic patients (OR=1.73; CI 95%=1.05-2.84).

Conclusions: These results suggest that the -308 polymorphism of the TNF- α gene contributes to CHD risk in type 2 diabetic population.

This work has been partially supported by FIS 99/0901, FIS 00/0757 and Fundació Marató TV3 (99/2010).

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Nucleotide (1385) T/G Polymorphism in Exon 2 of AdipoQ Is Associated with Coronary Artery Disease in Taiwanese

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Background and Aims: Adiponectin, the gene product of adipoQ, is an adipose-specific protein abundantly present in the circulation. It was observed to accumulate in the injured vascular walls, and was reported to have antiatherogenic properties. The aim of this investigation was to determine whether a previously known polymorphism in exon of adipoQ (1385 T/G) was related to coronary artery disease (CAD).

Materials and Methods: We examined the role of this polymorphism using a population association study. One hundred and twenty-eight angiography-proved CAD patients were recruited, and 202 normal controls (≥ 30 years old, BMI < 30) were randomly selected from health check-ups. Genotypes at this locus were determined by polymerase chain reaction restriction fragment length polymorphism. Differences between groups were evaluated by χ^2 test. Allelic frequencies were derived from the number of genotypes. Logistic regression analyses were applied to adjust the possible confounders.

Results: The distribution of genotypic frequency was different between the CAD and control groups ($p=.004$). The frequency of variant G was less in CAD subjects than in control (47.7% versus 63.9%, $p=.004$). Subjects with at least a G allele carried a protective effect from CAD by 0.515 times (95% confidence interval .328 to .808) as compared with those without a G allele. In multivariate logistic regression model, adjusted by age, sex, smoking, hypertension, BMI, and hyperlipidemia, this T/G polymorphism was still demonstrated to be a significant factor for CAD ($p=.016$).

Conclusions: The data suggest that the T/G polymorphism at the nucleotide 1385 of the adipoQ gene is associated with CAD in Taiwanese.

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LINKAGE DISEQUILIBRIUM BETWEEN APOC3 -455 T>C AND PREMATURE CORONARY HEART DISEASE IN INDO-MAURITIANS.

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Background and Aims: APOC3 is a constituent of triglyceride (TG) rich lipoproteins and is an important regulator of plasma TG concentrations. Several polymorphisms in the APOC3 gene have been previously associated with hypertriglyceridemia (HTG) and/or coronary heart disease (CHD). We studied the contribution of the 5' polymorphic nucleotide at position -455 T>C in the APOC3 gene promoter to premature CHD and components of the metabolic syndrome in subgroups of the Mauritian population.

Materials and Methods: We carried out case-control studies comparing two Indo-Mauritian groups of patients with premature CHD (age of onset before 60) with controls matched for ethnicity. We used PCR-RFLP techniques to study the APOC3 -455 T>C polymorphism in groups of the Mauritian population whose ancestors who migrated from the North (NI) or from the South of India (SI).

Results: Genotype proportions between TT, TC, CC were significantly different between male CHD NI patients (61,166, 67) and controls (36, 51, 34), Chi square=7.33, $p<0.03$. Genotype distribution was (6, 46, 22) in SI CHD patients v/s (8, 20, 17) in SI controls, Chi square=4.36, $p<0.11$. No association was found with abnormal glucose metabolism in either subgroup. The -455C variant was associated with HTG in the NI population ($p<0.008$). The -455T variant was associated with High Blood Pressure ($p<0.02$), and with the metabolic syndrome ($p<0.02$) in the SI population. The -455T variant was associated with central obesity ($p<0.02$) in both NI and SI male subgroups.

Conclusions: The -455 C>T variant is in strong linkage disequilibrium with CHD and influences components of the metabolic syndrome in Indo-Mauritians.

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THE FACTOR XIII CODON 34 VAL®LEU POLYMORPHISM DOES NOT PROTECT AGAINST MYOCARDIAL INFARCTION IN TYPE 2 DIABETIC PATIENTS WITH CORONARY ARTERY DISEASE

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Background and Aims: Factor XIII is a transglutaminase that crosslinks fibrin in the last step of the coagulation process. The factor XIII Val®Leu point mutation in codon 34 has been shown to protect against myocardial infarction in younger men. We aimed to clarify whether this protective effect is present in type 2 diabetic patients with coronary artery disease.

Materials and Methods: We studied 195 type 2 diabetic patients (140 males/ 55 females, age 63.5 ± 8.7 years) who underwent coronary angiography. The history of myocardial infarction was assessed by structured questionnaire, ECG and patient file. The factor XIII 34Val®Leu polymorphism was determined by PCR.

Results: The allele frequency of the factor XIII 34Leu variant was 24.4%, thus comparable to other Caucasian populations. The cardiac risk factors did not differ between the genotype groups. In 83.9% (68/81) of the patients with at least one Leu allele, coronary artery disease was found, compared to 81.6% (93/114) of those homozygous for the Val allele (odds ratio: 1.18; 95%-CI: 0.56-2.58; $p=0.67$). Among the patients with documented coronary artery disease, a history of myocardial infarction was found in 55.2% (37/67) among the patients with at least one Leu allele, and also in 55.2% (53/96) among those homozygous for the Val allele (odds ratio: 1.00; 95%-CI: 0.53-1.88; $p=1.00$). There was also no relation between the factor XIII genotype and the extent of coronary artery disease (odds ratio for three-vessel-disease for patients with at least Leu allele: 0.99; 95%-CI: 0.56-1.77; $p=0.99$).

Conclusions: The factor XIII codon 34 Val®Leu polymorphism does not appear to have any effect on presence or extent of coronary artery disease in type 2 diabetic patients, nor does it protect against myocardial infarction.

1198

COMBINED GENOTYPES ASSOCIATED WITH ACCUMULATION OF OXIDATIVE STRESS AND ATHEROSCLEROSIS IN DIABETIC SUBJECTS

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Background and Aims: We reported that somatic mutation rate of mitochondrial DNA (mtDNA) 3243 A to G might reflect accumulation of oxidative stress (Diabetes 49 suppl 1, 2000), which is a major etiology of atherosclerosis. NAD(P)H oxidase (NAOX) was an important source of reactive oxygen species (ROS), and renin-angiotensin II pathway was located at upstream of NAOX. However, it was unclear whether this pathway clinically may enhance ROS and develop atherosclerosis. Thus, the aim was to evaluate effects of combined genotypes of ACE and NAOX on accumulation of oxidative stress and early atherosclerosis in diabetic subjects. **Subjects and Methods:** In 268 Japanese type 2 diabetic subjects, genotypes of ACE I/D and NAOX subunit p22-phox C242T polymorphisms were analyzed by PCR-RFLP, and somatic mtDNA 3243G was examined by real time PCR using Taq Man probe, and carotid artery intima-media thickness (IMT) was measured by ultrasonography. **Results:** The subjects with the combination of genotypes (ACE: D(+) and p22-phox: T(-), $n=142$) showed higher mutation rate and mean IMT than the subjects with the other combination of genotypes ($n=126$) (0.0219 ± 0.0028 vs. 0.0097 ± 0.0012 % and 1.00 ± 0.03 vs. 0.91 ± 0.03 mm, means \pm SEM, both $p<0.01$). However, these were not different among the subjects with the combination except the above genotypes. **Conclusion:** Combination of ACE D(+) and p22-phox T(-) genotype may associate with accumulation of oxidative stress and early atherosclerosis in diabetic subjects.

1197

No Association of the Angiotensin-Converting-Enzyme Gene Insertion/Deletion Polymorphism with the Occurrence of Coronary Heart Disease in Patients with Diabetes Mellitus

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Aims: Previous studies have reported an association of the ACE-I/D-polymorphism with coronary heart disease (CHD) in patients with diabetes mellitus. However, ACE inhibitor treatment which could have compensated for negative effects of the D/D form of the ACE gene polymorphism was not considered in these studies. We therefore investigated the influence of the ACE-I/D polymorphism in patients with diabetes mellitus on the prospectively characterised occurrence of CHD by multiple regression analysis. **Methods:** Distribution of the ACE gene I/D-polymorphism was investigated in 400 patients with diabetes mellitus prospectively characterised for the presence/absence of coronary heart disease by coronary angiography and/or history for CHD (angina pectoris, dyspnea or myocardial infarction in the past)

Results: The distribution of DD; ID; II genotypes was 62 vs 114 vs 54 (26.96% vs 49.56% vs 23.48) in the CHD+ group and 33 vs 88 vs 49 (19.41% vs 51.76% vs 28.83%) in the CHD- group respectively ($p=0.17$). A multiple logistic regression analysis introducing the typical risk factors for CHD (age, gender, smoking, BMI >26 kg/m², LDL elevation, HbA1c $>7\%$) could not identify the ACE gene I/D-polymorphism as an independent risk factor for CHD ($p=0.09$).

Conclusion: Our data therefore suggest that the ACE gene I/D polymorphism is not associated with the occurrence of diabetic macroangiopathy especially in patients treated with ACE inhibitors. The treatment with ACE inhibitors may partially compensate for the negative effects of the D/D form and thus explain why our results are at variance with previous reports.

1199

ASSOCIATION OF THE GLU298ASP POLYMORPHISM OF ENDOTHELIAL NITRIC OXIDE SYNTHASE WITH CARDIOVASCULAR RISK IN TYPE-II DIABETES.

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Background and Aims: The Glu298Asp Polymorphism of the NO-synthase (NOS) is specified as a risk factor for coronary artery disease (CAD) in several studies. A reduced NO-biosynthesis based on an increased cleavage of the NOS is discussed as a reason. There are no specific investigations in patients with type-II diabetes regarding a possible contribution to CAD. The aim of this study was to investigate the role of this genetic variability in type II-diabetics in a case control study.

Patients and Methods: Investigations were performed in 214 type-II diabetics (69 ± 10 years, 67.8% male) with angiographically verified CAD and 109 control patients without clinically manifest CAD (66 ± 13 years, 49.5% male). The polymorphism of the NOS (EE:Glu/Glu, ED:Glu/Asp, DD:Asp/Asp) was analyzed using RFLP.

Results: As to be expected the classical risk factors age ($p<0.036$), male gender ($p<0.002$) and low HDL ($p<0.034$) were associated with CAD. The DD-genotyp was more frequent in controls than in diabetics with CAD (16.9% vs. 6.5%, $p<0.012$). Corrected for the above mentioned risk factors the odds ratio for CAD was 0.319 (95% CI: 0.135-0.755) for carriers of the DD genotyp.

Conclusions: In contrast to investigations in non diabetic populations, our results strongly suggest a reduced risk for CAD in type-II diabetic patients with the DD genotyp of the endothelial NOS.

1200

ANGIOTENSIN CONVERTING ENZYME GENOTYPE IS RELATED TO LIPID LEVELS IN TYPE 2 DIABETES AND INSULIN RESISTANCE SYNDROME

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Background and aims: Angiotensin-converting enzyme (ACE) insertion/deletion (I/D) gene polymorphism was reported to be associated with the presence of atherosclerosis both in general population and type 2 diabetes. The underlying mechanism for this association is unclear. The aim of the present study was to examine the effect of ACE I/D polymorphism on lipoprotein levels in subjects with type 2 diabetes or insulin resistance syndrome. **Materials and methods:** The study group included 99 patients (39 males and 60 females) with the mean age of 58±11 years. 66 patients had type 2 diabetes mellitus and 33 subjects had a dyslipidemia typical for insulin resistance (hypertriglyceridemia and/or decreased HDL cholesterol). ACE gene polymorphism was examined using PCR and RFLP methods. **Results:** Highly significant stepwise increase in apo B, LDL cholesterol and total cholesterol levels related to ACE genotype with the highest mean values in the group of DD homozygotes was observed. No significant difference in triglyceride or HDL cholesterol level was observed among the three genotypes. Since the difference between the II and ID groups was not significant, these two groups were pooled together and compared in further analyses with the DD-homozygote group (mean±SD shown in the table).

	II+ID (n=50)	DD (n=49)	P
Apo B (g/l)	1.01±0.25	1.20±0.23	0.0001
LDL-C (mmol/l)	3.03±1.18	3.56±1.03	0.0192
Cholesterol (mmol/l)	5.12±1.34	5.74±1.03	0.0114

Multivariate adjustments for age, sex, BMI, glycated hemoglobin and albuminuria did not change the significance of the relationship of the DD genotype to the lipid levels. **Conclusions:** In patients with type 2 diabetes or insulin resistance syndrome, homozygosity for D allele of ACE gene leads to increased levels of apo B and LDL cholesterol. In the Central European Caucasian population this might be one of the mechanisms underlying the association between the DD genotype and atherosclerosis in insulin resistant subjects.

1201

Diabetic microvascular complications are not associated with two polymorphisms in genes regulating glucose metabolism in Danish Type 1 diabetic patients

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Background and Aims: An XbaI polymorphism in the gene encoding the glucose transporter, GLUT-1, is associated with development of diabetic nephropathy in Chinese Type 2 diabetic patients. In addition, an amino acid variant (K121Q) in the gene encoding the glycoprotein plasma cell differentiating antigen (PC-1), a specific inhibitor of insulin receptor signalling, has been reported to predict a faster progression of nephropathy in Italian and British Type 1 diabetic patients.

Material and Methods: The XbaI and K121Q polymorphisms were determined by PCR-RFLP in Danish Type 1 diabetic patients with nephropathy (122 men/77 women, age 40.9 ± 9.6 years, diabetes duration 27 ± 8 years) and Type 1 diabetic patients with persistent normoalbuminuria (118 men/74 women, age 42.7 ± 10.2 years, diabetes duration 26 ± 9 years). Proliferative retinopathy was present in 156 patients (40%), while 67 patients (17%) had no diabetic retinopathy.

Results: There were no differences in the frequency of GLUT-1 XbaI genotypes between Type 1 diabetic patients with diabetic nephropathy and Type 1 diabetic patients with normoalbuminuria: 72 (41%) / 87 (50%) / 16 (9%) vs 94 (49%) / 74 (39%) / 24 (13%) had GLUT-1 XbaI +/+, +/- or -/- genotype, respectively (NS). The frequency of PC-1 KK, KQ and QQ genotypes were 141 (71%) / 52 (26%) / 6 (3%) vs 138 (73%) / 45 (24%) / 7 (4%) in patients with and without nephropathy, respectively (NS). No associations between the investigated polymorphisms and simplex or proliferative retinopathy were revealed either.

Conclusions: Neither the PC-1 K121Q, nor the GLUT-1 XbaI polymorphism contribute to the genetic susceptibility of diabetic microvascular complications in Danish Type 1 diabetic patients

PS 95

Risk Factors for Cardiovascular Complications

1202

Metabolic and Vascular Factors Contribute to Increased Cardiovascular Risk in HIV+ Patients Receiving Protease Inhibitors.

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Background and Aims: Insulin resistance and dyslipidemia, coexisting with central fat accumulation, are frequent findings in HIV+ patients treated with protease inhibitors (PI), and this constellation has been referred to as lipodystrophic syndrome (LDS). In HIV+ patients receiving PI we studied: 1. the prevalence of LDS; 2. the relationship between central fat accumulation and metabolic abnormalities; 3. endothelial function, measured by postischemic arterial vasodilation; 4. the effect of treatment with a slow-release bezafibrate preparation on severe dyslipemic patients. **Materials and Methods:** 53 subjects diagnosed as HIV+ (17 F, 36 M, aged 39 ± 2 years, BMI 26 ± 1 kg/m²; means ± SEM), without manifestations of AIDS, and treated with at least one PI drug, as well as 39 age, sex and weight matched healthy controls (CON) were studied. We evaluated BMI, fat tissue distribution, blood pressure (BP), fasting plasma glucose (FPG), serum insulin (INS), serum total cholesterol (CHOL) and triglyceride (TG) levels. Ischemia-induced vasodilation at the right a. brachialis was assessed by means of Doppler ultrasound. Insulin resistance was calculated using the HOMA approach. A subgroup of 18 patients received bezafibrate (400 mg/d for 2 months); lipid values were reassessed thereafter. **Results:** As compared to CON subjects, HIV+ patients showed higher FPG (91 ± 2 versus 82 ± 2 mg/dl), INS (17 ± 2 versus 9 ± 2 mU/L) and TG levels (284 ± 32 versus 110 ± 23 mg/dl; p < 0.05 for all parameters, Mann-Whitney's test). HOMA-IR index was 3.8 ± 0.7 and 1.9 ± 0.5 in HIV+ and CON subjects, respectively (p < 0.05). LDS was present in 34/53 patients; metabolic abnormalities were more severe in HIV+ patients with increased central fat deposition. Ischemia-induced vasodilation was impaired or absent in 74% of all HIV+ patients. No differences were detected in CHOL or BP levels between the two groups. In more severely dyslipemic subjects (basal TG 463 ± 51 mg/dl), a significant improvement in TG levels was observed after bezafibrate (203 ± 16 mg/dl; p < 0.0001, Wilcoxon's test). **Conclusions:** Our HIV+ patients display multiple metabolic CV risk factors; abnormal fat distribution is accompanied by more severe metabolic alterations, and the combination of somatic and metabolic changes (LDS) is present in 62%. Altered endothelial function may contribute to increased CV risk in these patients. Given the relevance of PI treatment for the survival of HIV infected individuals, the need for implementing therapeutic strategies that counterbalance these adverse metabolic/vascular changes is recognized. Bezafibrate proved to be an effective therapeutic approach to reduce hypertriglyceridemia in our patients.

1203

Screening for Cardiovascular Risk Factors in Employees of the Automobile Industries

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Background and Aims: Prevalence of diabetes mellitus, hypertension, hypercholesterolemia and adipositas of employees of an automobile company in Germany (Opel AG, Bochum).

Materials and Methods: 1547 (10%) of 13500 employees of Opel AG (GM) - Bochum were examined, 1423 (92.1%) male, 122 (7.9%) female, mean age 42.4 years.

Blood glucose, cholesterol, blood pressure, height and weight were measured. Diabetes mellitus is defined as an instantaneous blood glucose > 160 mg/dl, hypertension as a single systolic blood pressure of > 160 mm mercury, hypercholesterolemia as a total blood cholesterol of > 220 mg/dl and adipositas as a body mass index of > 25 kg/m². The measurements were compared with statements of a questionnaire.

Results: 3.8% of examined workers suffer from diabetes, 9 of them were newly diagnosed (15.3%). 27% are hypertensive, 38 of them were newly diagnosed (15.4%). 43.4% were hypercholesterolemic, 95 with new diagnosis (28.3%). 3.2% reported about diabetes, at least 3.8% were found to have diabetes. Similar results are given for hypertension with 22.3% vs 27%, hypercholesterolemia with 36.3% vs 43.4%, and adipositas with 61% vs 71%.

Conclusions: Even in a relatively young cross sectional cohort with socioeconomic importance a high prevalence for cardiovascular risk factors exists. The estimated number of undetected cases, reflected as the numbers of new diagnoses, is alarming.

1204

Prevalence of complications and cardiovascular risk factors in South Asian and European patients with onset of type 2 diabetes under age of 40 years

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Background and Aims: Our aim was to determine the prevalence of complications and cardiovascular risk factors at diagnosis of young onset type 2 diabetes, comparing Europeans and South Asians.

Materials and Methods: Patients: Newly diagnosed, prospectively screened 1998-2001. Diabetes <6 months, age 20-40, not ketosis prone, positive fasting insulin and C-peptide, good control off insulin for at least six months. Assessments: Vascular disease (previous infarct, angina, stroke, limb ischaemia), neuropathy (loss of 10g fine touch or vibration, or painful neuropathy), retinopathy (dilated fundoscopy), nephropathy (albumin creatinine ratio[ACR]), glycaemic control, BP, lipids, smoking, BMI, waist hip ratio (WHR), ECG, absolute CHD risk. **Results:** 292 patients (165 South Asian). Equivalent in age and sex. South Asians had more vascular disease (6.1% vs 3.1%, $p=0.01$), family history of vascular disease (52.1% vs 32.3%, $p<0.001$) family history of diabetes (41.2% vs 21.2%, $p<0.001$), and previous gestational diabetes in women (36.2% vs 16.3%, $p<0.001$). Similar numbers were smokers. South Asians had a slightly lower BMI (26.0 vs 27.2, $p=0.076$), significantly greater WHR (0.95 vs 0.90, $p=0.05$), trend to higher systolic BP (127 vs 123mmHg, $p=0.12$), more background retinopathy (13.9% vs 6.9%, $p<0.001$) and sight threatening retinopathy (3.6% vs 1.6%, $p<0.001$), higher prevalence of microalbuminuria (ACR men >2.5, women >3.5) (13.3% vs 5.5%, $p<0.001$) and macroalbuminuria (4.8% vs 2.3%, $p<0.001$). No difference in prevalence of foot problems, HbA1c, fasting plasma glucose or LDL cholesterol. Europeans had higher HDL cholesterol (1.3 vs 1.0mmol/L, $p<0.001$) and lower triglycerides (1.5 vs 1.9mmol/L, $p<0.001$). Mean absolute 10 year CHD risk was higher in South Asians (16.9% vs 13.7%, $p<0.001$), as was proportion of patients with CHD risk >30% (7.2% vs 4.7%, $p<0.001$) and >15% (17.5% vs 11.8%, $p=0.01$).

Conclusions: South Asians with young onset type 2 diabetes have significantly more complications at diagnosis, including established macrovascular disease, retinopathy and nephropathy than Europeans. South Asians also have a more atherogenic lipid profile (low HDL, raised triglycerides). Absolute CHD risk is also higher due to the lower HDL cholesterol and higher BP. Complications are common at diagnosis of young onset type 2 diabetes, and modifiable cardiovascular risk factors are also common.

1205

A comparison of haemorrhage and established risk factors to predict cardiovascular outcomes in patients with type 2 diabetes.

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Background and Aims: Abnormal haemorrhage has a pathogenic role in a wide range of vascular disorders. This retrospective study aimed to determine whether abnormal haemorrhage predicted cardiovascular [CV] morbidity and mortality over a 8-10 year period in people with type 2 diabetes, and to compare this predictive potential with other established CV risk factors. **Methods:** Baseline haemorrhageological data was obtained from 182 people with type 2 diabetes who had participated in 1 of 6 clinical studies carried out between 1988 and 1992 and included, whole blood viscosity and its determinants, haematocrit, plasma viscosity and plasma fibrinogen concentration. The following CV risk factors were recorded at baseline: Age, gender, smoking history, body mass index, duration of diabetes, glycaemic control, plasma lipid profile, plasma urate concentration, microalbuminuria and history of hypertension or previous CV disease. The occurrence over the next 8-10 years of myocardial infarction, stroke or death from a CV cause were then obtained from medical records. Univariate Cox regression analyses were used to identify individual risk factors showing an association with each clinical outcome [$P<0.10$]. Forward stepwise Cox regression analyses were then carried out to obtain a measure of the independent predictive strength of each risk factor. **Results:** Twenty-six patients with inadequate follow up data were excluded from the study. Univariate analyses of the data of the remaining 156 people showed that age, microalbuminuria, whole blood and plasma viscosity and plasma fibrinogen concentration >4.0g/L had an association with an increased risk of death from a CV cause. An unexpected finding in these univariate analyses was the absence of a significant association between mortality and established CV risk factors such as dyslipidaemia and body mass index. Stepwise regression analyses showed that age, microalbuminuria and increased plasma fibrinogen concentration were the only factors independently associated with death from a CV cause. Significant independent predictors for myocardial infarction or stroke were, age, HbA1c levels and pre-existing CV disease. **Conclusion:** Our findings are consistent with other studies that have shown microalbuminuria and plasma fibrinogen concentration are markers of increased CV risk in people with type 2 diabetes and perhaps of greater importance than other classic risk factors. Blood and plasma viscosity were not shown to be independent risk factors for any CV outcome in our study.

1206

LIPID PROFILE IN TYPE 2 DIABETES AND COEXISTING ISCHEMIC HEART DISEASE WITH OR WITHOUT EARLY RESTENOSIS AFTER CORONARY STENTING.

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Background and Aim: The role of disorders of lipid metabolism in the development of macroangiopathy and its complications is well known both in general population and type 2 diabetes. To assess alterations in lipid profile in patients with type 2 diabetes and coexisting ischemic heart disease (IHD) with (IHD-R) or without restenosis (IHD-NR) after coronary stenting (CS).

Subjects and Methods: Out of fifty-three consecutive IHD (39M/14 F; age median, range, 63, 43-74 yr; BMI, 27.0, 20-35.7 kg/m²) undergoing coronary angiography 6 months after CS, 24 (45.3%) had restenosis while 29 (54.7%) had not. In IHD or age- and weight-matched normal subjects (n=32, NS) blood levels of total-, HDL-, LDL-cholesterol, triglyceride, apoA, apoB, Lp(a) and HbA1c were determined.

Results: HDL-cholesterol was lower (mean±SEM, 45.9 ±2.0 vs. 60.0±3.0 mg/dl, $p<0.0001$) while triglyceride (160.0±10.6 vs. 103.4±7.7 mg/dl, $p<0.0002$) and LDL-cholesterol levels (134.0 ±4.9 vs. 133.1±6.9 mg/dl, $p=0.07$) were higher in IHD than in NS, respectively. Similar lipid alterations connoted both IHD-R and IHD-NR when compared separately with NS. HbA1c levels did not differ between IHD-R and IHD-NR (6.8±0.4 vs 6.6±0.3 %, $p=NS$). Moreover, there was no difference concerning age, BMI, frequency of hypertension and blood pressure levels between these two subgroups. Conversely, HDL cholesterol (42.8±2.5 vs. 48.4±2.9 mg/dl, $p=0.05$) as well as apoA (1.3±0.06 vs. 1.6±0.09 g/L, $p<0.009$) and Lp(a) (0.28±0.08 vs. 0.33±0.06 g/L, $p<0.04$) were lower in IHD-R than in IHD-NR.

Conclusions: An altered lipid profile is present in IHD patients and seems to be at higher risk for cardiovascular complications in those patients who develop restenosis six months after coronary stenting.

1207

HIGHER SERUM FERRITIN LEVELS IN DIABETICS WITH ANGIOGRAPHICALLY ASSESSED CORONARY ATHEROSCLEROSIS

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Background and Aims: Elevated serum ferritin concentrations in CVD patients have been reported by several studies. Ferritin levels are generally considered as a good marker of body iron stores. Iron bound to ferritin may contribute to the oxidative modification of LDL and thus enhance their atherogenicity. Aim of the study was: 1. Comparison of serum ferritin concentrations in diabetics vs. non-diabetics in the cohort of patients with angiographically assessed coronary vascular disease. 2. Correlation of ferritin with certain markers of oxidative stress and other variables.

Materials and Methods: Measurements were carried out in 48 men (mean age±SD of diabetics/non-diabetics: 61.5±8.87 / 61.4±8.66 ys) and 16 women (mean age±SD of diabetics/non-diabetics 61.5±10.18 / 62.1±9.38 ys). Ferritin, antibodies against oxidized LDL (anti-oxLDL), total cholesterol and triacylglycerols (TG) were estimated by enzyme immunoassay; HDL-cholesterol with PEG-modified direct measurement; nitrites/nitrates were measured by method based on enzymatic reduction of nitrates by nitrate reductase and subsequent estimation of nitrites by Griess' reaction; oxidized LDL (oxLDL) in serum were estimated by direct spectrophotometric measurement; lipophilic vitamins A and E were determined by HPLC chromatography. All diabetic patients had type 2 DM. Left ventricle ejection fraction was measured by B-mode ultrasonography. The comparison of the diabetics vs. nondiabetics was performed in age- and sex-matched groups.

Results: CVD patients with diabetes mellitus had significantly higher serum ferritin concentrations than nondiabetics (157,40±57,92 vs. 132,43±37,74 mg/ml, $t=1,5469$, $p<0,05$). Nitrites/nitrates were significantly lower in diabetics (22,49±5,85 vs. 18,11±3,89 mmol/L, $t=1,7535$, $p<0,05$). In the whole group of CVD patients, ferritin correlated positively with total cholesterol ($p<0,01$), LDL-cholesterol ($p<0,05$), TG ($p<0,05$), creatinine ($p<0,01$) and retinol ($p<0,05$); there was a negative correlation of ferritin with left ventricular ejection fraction ($p<0,01$).

Conclusions: 1. Among CVD patients, type 2 diabetics showed higher serum ferritin concentrations than nondiabetics. 2. Higher serum ferritin concentrations were associated with lower left ventricular ejection fraction in CVD patients. 3. The correlation of ferritin with total cholesterol, LDL-cholesterol and creatinine may reflect a higher consumption of meat and meat products (containing heme iron) in diabetic patients.

Supported by grant of Ministry of Health 6124/3

1208

Plasma leptin and the risk of cardiovascular disease in the West of Scotland Coronary Prevention Study

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Background and Aims: Leptin plays a role in fat metabolism and obesity, and correlates with insulin resistance and other markers of the metabolic syndrome independent of total adiposity. Raised leptin levels also appear to predict those at risk for diabetes. In light of these observations, we hypothesised that raised plasma leptin levels may identify men at increased risk of a coronary event in the West of Scotland Coronary Prevention Study (WOSCOPS).

Materials and Methods: Plasma leptin was measured at baseline in 377 men (cases) who subsequently suffered a myocardial infarction, sudden coronary death or underwent coronary revascularisation, and 783 men (controls) who remained free of a coronary events during the 5 year follow up period of the study. Controls were matched to cases on the basis of age and smoking history and were representative of the entire WOSCOPS cohort.

Results: Leptin was significantly higher in cases than controls (mean (SD) of 5.87 [2.04] mg/l versus 5.04 [2.09] mg/l, $P < 0.001$). In univariate analysis, for each 1 SD increase in leptin the relative risk (RR) of an event increased by 1.25 [95% confidence interval (CI): 1.10-1.43, $P = 0.0006$]. There was minimal change in this RR with correction for BMI [RR=1.24 (CI: 1.06-1.45), $P = 0.0062$] or with further correction for traditional risk factors including age, BMI, lipids, BP [RR=1.21 (CI: 1.02-1.42) $P = 0.02$]. Leptin was correlated with CRP ($r = 0.24$, $P < 0.001$) but even with this variable in the model, leptin retained borderline significance as a predictor of coronary events [RR=1.18 (CI: 1.00-1.39) $P = 0.05$].

Conclusions: In conclusion, we have shown for the first time in a large prospective study that plasma leptin concentration is a novel independent risk factor for CHD.

1209

Risk factors associated with peripheral vascular disease of low extremities among type 2 diabetic patients in Taiwan

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Background and Aims: To find risk factors associated with peripheral vascular disease (PVD) of low extremities among type 2 diabetic patients.

Materials and Methods: From July 1996 to June 2000, there were 1,140 male and 1,139 female type 2 diabetic patients, 40 to 79 years old, with mean (\pm SD) duration of diabetes 9.71 (\pm 8.07) years, screened for PVD by Doppler ultrasound at metabolic clinic of National Taiwan University Hospital. Ankle-brachial index below 0.9 was deemed as PVD.

Results: The prevalence of PVD among type 2 diabetic patients was 7.0% and the mean age and duration of diabetes were significantly higher in those with PVD. After adjusting age, gender and duration of diabetes, several risk factors were found to be associated with increased risk of PVD including systolic blood pressure, hemoglobin A1c, triglyceride, total cholesterol and cigarette smoking. The odds ratio increased with increasing systolic blood pressure (p for trend = 0.000) and the odds ratio (OR) was 3.37 (95% CI=1.99 – 5.69) for systolic blood pressure greater than 145 mmHg. An almost 50% increase in risk of PVD were found among those with hemoglobin A1c more than 7.0%, triglyceride greater than 200 mg/dl and total cholesterol greater than 200 mg/dl (OR=1.57, 95% CI=1.07 – 2.29, OR=1.50, 95% CI=1.07 – 2.10 and OR=1.55, 95% CI=1.08 – 2.22, respectively). Cigarette smoking was also found to be associated with an almost two-fold increased risk of PVD (OR=1.82, 95% CI=1.08 – 3.07). In a multivariate model with above variables, higher level of triglyceride, systolic blood pressure greater than 145 mmHg and cigarette smoking were still associated with increased risk of PVD.

Conclusions: More rigorous control of blood pressure, dyslipidemia and cessation of smoking is indicated to decrease the risk of PVD.

PS 96

Inflammation and Cardiovascular Complications

1210

Relationships between albumin excretion rate, inflammatory markers and platelets in type 2 diabetic subjects.

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Background and Aims: Albumin excretion rate (AER) is a marker of endothelial dysfunction and predicts cardiovascular mortality in the general population and in type 2 diabetic subjects. Aim of the present study was to evaluate, in type 2 diabetic patients, the relationship between AER and two haematologic parameters that have been considered as predictors of vascular damage, i.e. leukocyte count and mean platelet volume (MPV), the first one being an inflammatory marker, the second a marker of a prothrombotic state.

Materials and Methods: Patients ($n = 659$, M/F=354/305) were in regular follow-up at our outpatient clinic. Population characteristics (mean \pm SD): age, 62.00 \pm 9.48 years; known diabetes duration, 9.59 \pm 8.21 years; BMI, 29.04 \pm 4.94 Kg/m²; AER(log), 1.13 \pm 0.61 μ g/min; fibrinogen, 337.88 \pm 79.87 mg/dl; hematocrit (HT), 41.4 \pm 3.7%; erythrocyte sedimentation rate (ESR), 22.54 \pm 17.7 mm/h; white blood cells (WBC), 7028.8 \pm 1782.2 μ l; platelet count (PLTS), 231.1 \pm 62.1 μ l; MPV, 9.6 \pm 1.3 fl.

Results: Parameters were divided according to values of AER (< 20 , 20-200 $e > 200$ μ g/min) and analyzed by ANOVA: age, $p = ns$; disease duration, $p = 0.001$; BMI, $p = ns$; fibrinogen, $p < 0.0001$; HT, $p = ns$; ESR, $p = 0.0001$; WBC, $p = 0.0023$; PLTS, $p = ns$; MPV, $p = ns$. By multiple regression analysis of AER (log) vs 17 variables (age, diabetes duration, BMI, systolic blood pressure, diastolic blood pressure, HbA1c, TC, HDL-C, TG, creatinine, uric acid, fibrinogen, ESR, haematocrit, WBC, PLTS and MPV) the values were: haematocrit, std coeff=0.032, $p = ns$; fibrinogen, std coeff=0.103, $p = 0.0129$; ESR, std coeff, 0.058, $p = ns$; WBC, std coeff=0.133, $p = 0.0011$; PLTS, std coeff=-0.101, $p = 0.0164$; MPV, std coeff=-0.090, $p = 0.0186$. If only subjects without previous cardiovascular events ($n = 544$) were considered in multiple regression analysis, the relationship of AER (log) with WBC was maintained (std coeff=0.161, $p = 0.0003$). If WBC and fibrinogen were not included in the analysis, PLTS and MPV were no longer significantly related to AER (log): std coeff=-0.02, $p = ns$ for PLTS and std coeff=-0.056, $p = ns$ for MPV.

Conclusions: In type 2 diabetic patients, AER, a marker of endothelial dysfunction, is positively associated with WBC number, also in patients without previous cardiovascular events; MPV is not correlated with AER per se, but, opposite to what one could expect, becomes negatively correlated with AER when inflammatory markers (namely fibrinogen and WBC) are incorporated in multiple regression analysis. This observation could suggest that, when endothelial dysfunction is present, there is an increased consumption of bigger, more reactive platelets.

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C-REACTIVE PROTEIN IS INCREASED IN TYPE I DIABETIC PATIENTS AND CORRELATES WITH MARKERS OF ENDOTHELIAL DYSFUNCTION

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Background and Aims: endothelial dysfunction and inflammatory response play an important role in the development of diabetic vascular complications. The aim of the study was to estimate the concentration of C-reactive protein (CRP), endothelin -1 (ET-1), metabolites of nitric oxide (NO₂), Interleukin Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1) and the level of von Willebrand factor (vWF) in Type I diabetic patients. Moreover, we studied the relationship between serum CRP and markers of endothelial dysfunction. **Materials and Methods:** the study was performed in 67 Type I diabetic patients (39 women and 28 men, aged 31.7 \pm 9.2 years, duration of disease 17.4 \pm 6.2 years and HbA1c 8.5 \pm 2.1 %). Serum concentration of CRP, ET-1, NO₂, ICAM-1, VCAM-1 and level of vWF were estimated with the use of the ELISA tests. **Results:** in comparison with healthy subjects we observed significantly higher values of CRP, ICAM-1, VCAM-1, ET-1, NO₂ and vWF. The serum CRP concentrations positively correlate with assessed markers of endothelial dysfunction.

Parameter	Healthy subjects	Diabetic patients	p	r
CRP (mg/l)	1.10 \pm 0.07	4.91 \pm 0.28	$p < 0.001$	-
ET-1 (pg/ml)	0.60 \pm 0.05	2.35 \pm 0.24	$p < 0.002$	0.58
NO ₂ (μ mol/l)	16.68 \pm 0.48	85.49 \pm 0.24	$p < 0.05$	0.86
sICAM-1 (ng/ml)	210.60 \pm 7.16	241.42 \pm 9.12	$p < 0.05$	0.60
sVCAM-1 (ng/ml)	553.02 \pm 10.50	696.82 \pm 30.53	$p < 0.05$	-0.20
vWF (%)	110.00 \pm 3.96	156.92 \pm 4.20	$p < 0.05$	0.47

Mean \pm SEM, r - Pearson correlation coefficient

Conclusions: the results obtained might suggest that the inflammatory process is strongly associated with endothelial dysfunction in diabetes.

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Plasma and Urinary Cytokine Antagonists in Type 1 Diabetes Mellitus

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Background and Aims: Several cytokines and growth hormones have been implicated in the pathogenesis of diabetic nephropathy. Their ability to generate a biological response in vivo is modulated by specific antagonists and soluble receptors. The aims of the study were a) to measure the levels of interleukin 1 receptor antagonists (IL-1ra) and tumor necrosis factor alpha soluble receptors p55 (TNFsr1) and p75 (TNFsr2) in plasma and urine and b) to test their response to acutely-induced hyperglycaemia and furosemide administration in type 1 diabetes mellitus (DM1).

Materials and Methods: Plasma concentrations and urinary excretions of IL-1ra, TNFsr1 and TNFsr2 were measured in two 90-minute periods of glycaemic clamp-induced eu- and hyperglycaemia (5 and 12 mmol/l, Study 1) during time-controlled euglycaemia (Study 2) and before and after iv. furosemide (0.5 mg/kg) administration in 20 DM1 patients with normal albumin excretion and normal glomerular filtration rate, and in 15 weight-, age- and sex-matched healthy controls (C).

Results: Plasma concentrations of IL-1ra (215±98 vs 194±59 pg/ml), TNFsr1 (838±207 vs 794±311 pg/ml) and TNF sr2 (2538±421 vs 2447±378 pg/ml) were comparable in DM1 and C, and no significant changes during Study 1, Study 2 or after furosemide were found. The urinary IL-1ra excretion was significantly higher in DM1 compared to C (8259±8401 vs 1586±1123 pg/min; $p<0.05$), during euglycaemia. In DM1 it significantly decreased during Study 1, compared to Study 2 ($p<0.05$), while it did not change in C. The urinary excretions of TNFsr1 and TNFsr2 were comparable in DM1 and C. In DM1 patients TNFsr1 significantly declined after furosemide (1437±902 vs 768±589 pg/min; $p<0.01$) and during Study 1 (1957±1387 vs 925±450 pg/min; $p<0.001$) compared to Study 2 (1522±730 vs 1263±618 pg/min; $p<0.01$). Similarly, TNFsr2 decreased after furosemide (2842±1475 vs 1758±1034 pg/min; $p<0.01$) and during Study 1 (2827±1934 vs 1761±766 pg/min; $p<0.01$). Hyperglycaemia and furosemide did not change TNFsr1 and TNF sr2 urinary excretions in C.

Conclusions: DM1 with normal renal haemodynamics is associated with impaired regulation of renal IL-1ra, TNFsr1 and TNFsr2 production with possible impact on local control of cytokine activity in kidneys. (Supported by IGA MZ CZ grant NB/6365-3).

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Chronic, subclinical inflammation and improved metabolic control in type 2 diabetic patients.

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Background and Aims: An association of chronic, subclinical inflammation with an increased cardiovascular risk has been shown and diabetic patients present with high circulating levels of acute phase proteins. We studied the impact of improved metabolic control on concentrations of C-reactive protein (CRP), fibrinogen (FIB), interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1) and tumor necrosis factor alpha (TNF) in patients with type 2 diabetes. **Materials and Methods:** This is a prospective study in poorly controlled (Mean (SD) HbA1c: 9.1 % (1.3)) type 2 diabetic patients (n=26, age (yrs): 58.7 (7.1)), who underwent a structured educational program, primarily aiming at improving dietary habits. Clinical visits were at weeks 4, 12, and 24. Inflammatory proteins were measured using highly sensitive immunoassays, all other measures were performed using standard methods. Oral hypoglycemic therapy remained unchanged during the study period. Longitudinal differences were calculated using the paired t-test, and Pearson's correlation analyses were performed. **Results:** After 12 weeks metabolic control improved markedly (HbA1c (%): 9.1 (1.3) vs. 7.2 (1.4), $p=0.0001$), as well as measures of body fat and body fat distribution (body weight (kg): 86.7 (13.6) vs. 83.8 (13.8), $p=0.0001$; BMI (kg/m²): 31.0 (5.5) vs. 29.9 (5.3), $p=0.0003$; waist circumference (cm): 102.0 (12.0) vs. 98.9 (13.5), $p=0.023$). However, inflammatory proteins remained unchanged (CRP (mg/L): 5.05 (3.8) vs. 4.72 (3.7); TNF (pg/ml): 7.24 (6.5) vs. 8.44 (8.9); IL-6 (pg/ml): 4.73 (2.8) vs. 4.87 (3.5)), with a tendency towards a decrease for FIB (mg/dl: 397 (86) vs. 383 (92), $p=0.15$) and PAI-1 (U/ml: 27.7 (14.8) vs. 23.1 (12.6), $p=0.08$). We found significant inter-relationships among inflammatory proteins (CRP vs. FIB: $r=0.65$, CRP vs. IL-6: $r=0.48$, CRP vs. TNF: $r=0.42$, FIB vs. TNF: $r=0.43$, IL-6 vs. TNF, $r=0.44$, all p -values <0.05). PAI-1 was related to components of the insulin resistance syndrome (vs. BMI: $r=0.55$, vs. subscapular skinfold: $r=0.48$, vs. triglycerides: $r=0.75$, vs. serum insulin: $r=0.55$, all p -values <0.05). **Conclusions:** A significant improvement of metabolic control (HbA1c: minus 1.8%) was not associated with a decrease in circulating inflammatory proteins in type 2 diabetic patients within 12 weeks. These data support the notion - as derived from the UKPDS - that improvement of metabolic control alone may not suffice to improve the increased risk of macroangiopathy in type 2 diabetic patients.

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Fibrinogen and C-Reactive Protein levels in patients with diabetes mellitus: Association with complications

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Background and Aims: Recent studies show that in diabetic subjects an increase of plasma fibrinogen and C-reactive protein (CRP) concentrations are associated with a high risk of cardiovascular complications. The aim of this study was to investigate the plasma fibrinogen and CRP levels and their relationship with diabetic complications in patients with Type 2 diabetes mellitus (DM).

Materials and Methods: 73 patients with DM and age and sex matched 23 healthy controls were included to the study. Duration of diabetes mellitus, body mass indexes, plasma glucose, HbA1c, lipid profiles, fibrinogen and CRP levels were determined.

Results: Both plasma CRP and fibrinogen levels were found to be significantly higher in patient group than control group (8.28±/ 6.96 vs. 3.42 ±/ 0.73 mg/L, 4.29 ±/ 1.24 vs. 3.33±/ 0.66 g/L respectively, $p<0.01$ for both). Patients without any detected diabetic complications also showed increased plasma fibrinogen and CRP levels when compared to controls (7.02±/ 6.639 vs. 3.42 ±/ 0.73 mg/L, 3.93 ±/ 0.75 vs. 3.33±/ 0.66 g/L respectively, $p<0.01$ for both). When patients were re-evaluated according to their microvascular complications no difference could be found, regarding CRP and fibrinogen levels, between patients groups with or without nephropathy, retinopathy or neuropathy. On the other hand, patients with clinical macrovascular disease (n=22) showed significantly increased CRP levels than both controls (10.64±/ 7.34 vs. 3.42 ±/ 0.73 mg/L $p<0.001$) and patients without clinical macrovascular disease (n=51, 7.26 ±/ 6.6 vs. 3.42 ±/ 0.73 mg/L $p<0.01$). 50% of the patients with macrovascular disease had clinically increased CRP levels (>10 mg/L) and 81.8 % had elevated fibrinogen concentrations. There was a negative correlation between HDL-cholesterol and CRP levels ($r=-0.431$, $p<0.01$). None of the other parameters showed correlation with plasma fibrinogen or CRP levels.

Conclusions: The data suggest that Type 2 diabetes mellitus is associated with a systemic inflammatory response. Fibrinogen and C-reactive protein concentrations are higher in diabetic patients. As a marker of systemic inflammation, raised CRP concentrations are associated with an increased incidence of macrovascular disease. Chronic inflammation therefore emerges as a potential mediator of macrovascular disease. These findings may be relevant to strategies aimed at reducing risk of macrovascular complications in patients with diabetes.

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ALBUMINURIA, ENDOTHELIAL DYSFUNCTION AND INFLAMMATION ARE ASSOCIATED WITH RISK OF DEATH IN TYPE 2 DIABETES

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Background and Aims: In type 2 diabetes, an increased urinary albumin excretion (AER) is strongly associated with risk of death. The nature of this association is poorly understood, but it is unlikely to be causal. We investigated whether chronic, low-grade inflammation and/or endothelial dysfunction can explain the association between increased AER and risk of death in type 2 diabetes. **Materials and Methods:** We followed a cohort of 328 patients with type 2 diabetes prospectively with repeated measurements of AER and plasma markers of chronic, low-grade inflammation (C-reactive protein, fibrinogen) and endothelial dysfunction (von Willebrand factor, tissue-type plasminogen activator, soluble E-selectin, soluble vascular cell adhesion molecule-1). We assessed their associations with mortality as well as their mutual relationships. **Results:** After a mean duration of follow-up of 9.0 (range 0.2 to 10.9) years, 113 (34%) patients had died. After adjustment for cardiovascular risk factors, AER and plasma levels of C-reactive protein and soluble vascular cell adhesion molecule-1 were significantly and mutually independently associated with mortality. The longitudinal development of AER was significantly and independently determined by baseline levels of, and the longitudinal development of BMI, systolic BP, s-creatinine, HbA_{1c} and plasma von Willebrand factor (baseline only), soluble E-selectin (baseline only), tissue-type plasminogen activator, C-reactive protein, and fibrinogen. The longitudinal developments of markers of inflammation and of endothelial function were interrelated, but neither clearly preceded the other. **Conclusions:** Endothelial dysfunction and chronic, low-grade inflammation in type 2 diabetes are determinants of an increased AER but do not explain why an increased AER is associated with mortality. Chronic, low-grade inflammation, endothelial dysfunction and increased AER are interrelated processes, develop in parallel, and are strongly but independently associated with risk of death.

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C-Reactive Protein Is Elevated In Type 1 Diabetic Women And Is Associated With Coronary Artery Calcification.

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Background and Aims: Whether C-reactive protein (CRP), marker of systemic inflammation, is elevated in type 1 diabetes is not clear. Our aim was to examine whether CRP is elevated in type 1 diabetic patients and whether it is associated with coronary artery calcification (CAC) a measure of atherosclerosis.

Materials and Methods: Using electron beam computed tomography, CAC was quantified in 194 men and 200 women with (50%) and without type 1 diabetes aged 30-50 yrs randomly sampled from diabetic clinics and the general population, respectively. CRP was measured using a highly sensitive immunoassay.

Results: CRP was greatly elevated in women with diabetes (median CRP 2.78 mg/L) compared to non-diabetic women (median CRP 1.62 mg/L, $p=0.003$). In contrast there little difference in CRP between diabetic (median CRP 1.70 mg/L) and non-diabetic men (CRP 1.4 mg/L, $p=0.6$). The elevation in CRP in diabetic women was independent of BMI, waist hip ratio, physical activity, blood pressure and lipids, all of which were associated with CRP ($p=0.009$ on adjustment for these). Among diabetic subjects CRP was positively associated with HbA1c (Spearman's correlation coefficient $r=0.25$, $p=0.0004$) but HbA1c did not explain the greater elevation of CRP in diabetic women than men. CAC prevalence was similar in diabetic (52%) and non diabetic (52%) men. Among women, CAC was much more prevalent in those with diabetes than without (47% vs. 21%, Odds ratio =3.6, 95% CI:2-7, $p<0.0001$ adjusted for age). In all subjects combined those with detectable CAC had higher levels of CRP (median 0.7 mg/L) than those without (1.5 mg/L, $p<0.001$ adjusted for age sex and diabetes). This association was independent of lipids, BP, BMI and waist hip ratio ($p=0.03$ on adjustment) and was apparent in all four diabetes sex groups.

Conclusions: CRP is selectively elevated in diabetic women and is associated with coronary atherosclerosis. This suggests that the role of inflammation in the increased risk of coronary disease in diabetic women deserves further investigation.

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TNF-alpha inhibits insulin-mediated vasodilatation in rat skeletal muscle.

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Background and Aims: Abnormal reactivity of resistance vasculature may contribute to the pathogenesis of diabetic microangiopathy, inducing alterations in regional hemodynamics. Tumor necrosis factor-alpha (TNF-alpha), an important inflammatory cytokine, may be implicated as causative factor in the pathogenesis of type 2 diabetes. The aim of our study was to determine 1) the hemodynamic effect of insulin at the level of microcirculation and, 2) the impact of TNF-alpha on insulin reactivity.

Materials and Methods: In fasted anesthetized rats, the spinotrapezius muscle was exteriorized and superfused with isotonic buffer for in situ visualisation of the microcirculation by intravital microscopy. The reactivity of the arterioles ($<20 \mu\text{m}$) to insulin (1 IU/kg and repeated injections of 0.25 IU/kg every 15 min; S.C.) was determined in the presence or not of an intravenous 2h-TNF perfusion (0.5 $\mu\text{g/h}$). At the end of the study, the skeletal microvascular blood flow (MBF) was assessed by a soluble marker, the 3H desmethylimipramine (75 $\mu\text{Ci/kg}$; I.V.).

Results: Hyperinsulinemia caused a significant increase in MBF (1.97-fold) and arteriolar diameter (15%). This vasodilatation was already present at time 15 min and persisted until the end of the experiment. TNF-alpha completely prevented the insulin-mediated precapillary arteriolar vasodilatation and MBF elevation. TNF alone had no significant effect on these variables.

Conclusions: Our data show that hyperinsulinemia induced precapillary arteriolar vasodilatation. The acute in vivo administration of TNF-alpha completely abolished the hemodynamic action of insulin in rat spinotrapezius muscle. This finding suggest that TNF-alpha may be one of the factors contributing to vascular dysfunction in diabetic patients.

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Soluble tumor necrosis factor-alpha receptors in young adults with type 1 diabetes: relations to smoking and microvascular complications

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Background and Aims: The purposes of this study were 1) to compare plasma levels of soluble receptors of tumor necrosis factor-alpha (sTNF-R), which are thought to reflect the degree of activation of the TNF-alpha system, in nondiabetic subjects and type 1 diabetic individuals, and 2) to evaluate the effects of chronic smoking and microvascular complications on plasma sTNF-R levels in type 1 diabetic individuals.

Materials and Methods: Plasma levels of two sTNF-R (sTNF-R1 and sTNF-R2) were measured in 50 young type 1 diabetic patients without clinical evidence of macroangiopathy and in a matched-group of 20 healthy volunteers.

Results: When diabetic patients were subdivided according to smoking status and microvascular complications (i.e., microalbuminuria and/or retinopathy), the subgroups of patients had similar values of age, sex, BMI, blood pressure, lipids, creatinine and glycometabolic control. Nevertheless, plasma levels of sTNF-R1, but not of sTNF-R2, were markedly elevated ($p<0.05$ or less) in complicated vs uncomplicated (2.40 ± 0.3 vs 1.80 ± 0.1 ng/ml) patients and in smokers vs nonsmokers (2.66 ± 0.4 vs 1.76 ± 0.1 ng/ml). When diabetic patients were categorized for the number of cigarettes smoked daily, the relationship between plasma sTNF-R1 and smoking was strictly dose-dependent. Similarly, when patients were graded for the degree of nephropathy and retinopathy, plasma levels of sTNF-R1, but not of sTNF-R2, were significantly higher in patients with microalbuminuria vs normoalbuminuria, and in patients with severe retinopathy compared with those with background retinopathy or with no retinopathy. In a 2-factor analysis of variance, both smoking ($p<0.01$) and microvascular complications ($p<0.05$) were independent predictors of plasma sTNF-R1. Plasma sTNF-R1 and sTNF-R2 concentrations of diabetic patients who did not smoke or without complications were substantially similar of those of healthy controls.

Conclusions: Chronic smoking and microvascular complications can exert an additive and deleterious impact on the activation of TNF-alpha system, as reflected by the levels of circulating sTNF-R, in young adults with type 1 diabetes.

PS 97 Glycation

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Advanced glycosylation end products increased expression of monocyte chemoattractant protein-1 in human umbilical vein endothelial cells.

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Background and Aims: Accumulation of advanced glycosylation end products (AGEs) was found in atherosclerotic lesions and may play an important role in pathogenesis of atherosclerosis. Monocyte chemoattractant protein-1 (MCP-1) is expressed in many type of cells and also in atherosclerotic plaques. Therefore, MCP-1 is thought to be involved in atherogenesis. This study is designed to investigate effect of AGEs on expression of MCP-1 in human umbilical vein endothelial cells (HUVEC).

Materials and Methods: AGEs was prepared by incubation of BSA with different concentrations of glucose for different periods. Expression of MCP-1 mRNA in cultured HUVEC exposure to AGEs was quantified by RT-PCR. MCP-1 protein was determined by western blotting. Biological activity of MCP-1 was observed by monocyte chemotaxis assay performed in a modified Boyden chamber.

Results: When the cells were incubated with AGEs-BSA (200 mg/L) glycosylated with glucose of different concentrations (0, 20, 50, 80 mmol/L) for 24 h, expression of MCP-1 mRNA and MCP-1 protein in HUVEC and distance of monocyte migration were increased significantly with ascent of concentrations of glucose, with a peak at the glucose concentration of 50 mmol/L (mRNA 0: 0.86 ± 0.03 ; 20: 0.88 ± 0.02 ; 50: 1.08 ± 0.03 ; 80: 0.93 ± 0.01 ; protein 0: 10.92 ± 1.1 ; 20: 20.89 ± 2.91 ; 50: 34.05 ± 2.98 ; 80: 28.95 ± 3.11 ; distance 0: 28.8 ± 4.23 ; 20: 30.4 ± 5.11 ; 50: 45.0 ± 3.84 ; 80: 41.0 ± 2.51). When the cells were treated with AGEs-BSA (200 mg/L, glucose 50 mmol/L) for different periods (0, 12, 24, 36 h), expression of MCP-1 mRNA and protein in HUVEC and migration distance were increased with time of AGEs incubation, with a peak at 24 h. When the conditioned medium of HUVEC produced by AGEs was pretreated by anti-MCP-1 antibody, increased distance of monocyte migration was not found.

Conclusions: AGEs enhanced expression of MCP-1 in HUVEC and followed by increased chemotaxis activity for monocyte.

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ADVANCED GLYCATION ENDPRODUCTS IN HUMAN DIABETIC PERIPHERAL NERVE

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Background and Aims: Nonenzymatic glycation leading to AGE formation is one of the important biochemical pathways involved in the development of long-term diabetic complications and with a potential role in diabetic neuropathy. The aim of our study was in situ detection of AGE-immunoreactivity in the sural and femoral nerve from type 2 diabetic patients with proximal diabetic neuropathy (PDN).

Materials and Methods: Twelve patients with classic, abrupt-onset, sensorimotor form of PDN were included in the study (age 65.0 ± 6.6 yrs, m/f 7/5, diabetes duration 12.5 ± 5.6 yrs, PDN duration 4.2 ± 1.7 mo, HbA1c 7.7 %). The specimens were collected by biopsy of a small sensory branch of the femoral and sural nerves and AGE deposits were detected by an indirect immunofluorescence technique. The primary antibody was polyclonal anti-AGE. Competitive ELISA was used to measure total serum AGEs and blocking ELISA to measure anti-AGE autoantibodies. Soluble AGE-immune complexes were detected by immunohistochemical method.

Results: Immunohistochemical examination under fluorescence microscope demonstrated AGE deposits located in the perineurium, focally in the endoneurium, and in the myelin protein area of diabetic nerves. A strong AGE positivity was detected in five out of six femoral nerve samples and was located mainly in the myelin sheath. In sural nerve sections AGE deposition was found in the myelin, perineurium, and focally in the endoneurium. Six of them showed positivity in all the patterns, while in one sural nerve section AGE deposits appeared in the perineurium only. The AGE-immunoreactivity in the control specimen was negative or of very low intensity. Total AGEs, free anti-AGE antibodies and circulating AGE-IC were significantly higher in diabetic ($n=12$) than in control ($n=20$) serum samples (AGEs: 38.6 ± 6.9 vs. 25.1 ± 7.2 ugEq/ml, $p < 0.001$; Anti-AGE antibodies 58.7 ± 20.2 vs. 17.4 ± 15.4 AU, $p < 0.001$; AGE-IC 5.9 ± 2.4 vs. 3.39 ± 1.1 AU; $p < 0.001$). **Conclusions:** Our study demonstrated excessive AGE deposition on peripheral nerve cytoskeletal and myelin protein components, and significantly higher circulating AGE-immune complexes in human diabetic neuropathy.

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EFFECTS OF DIETARY CARBOXYMETHYLLYSINE (CML) INTAKE ON CML LEVELS AND ALBUMIN EXCRETION RATES IN HEALTHY SUBJECTS

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Background and Aims: Advanced glycation end products (AGEs), particularly CML, have been implicated in diabetic complications. Besides endogenous formation, AGEs can derive from food as well. The aim of this study was to elucidate whether dietary CML-load exerts detrimental effects on renal function in healthy subjects. **Materials and Methods:** A cross-over study was carried out in 21 young adults (age: 25 ± 5 years). We compared the effect of heated (CML-rich) foods to the effect of unheated (CML-poor) foods on serum and urine CML levels (ELISA, Roche Diagnostics, Penzberg, Germany), 24-hour creatinine clearance, AER and blood pressure. Protein content of diet was $3g \times kg$ body weight $^{-1} \times day^{-1}$. Heated diet contained approx. 60 mg CML/day, while CML content of unheated food was negligible. Volunteers were randomised into 2 groups. After a baseline week with habitual diet subjects in group A consumed CML-rich food for one week and after another week with habitual diet they consumed CML-poor diet for the last week. In group B the sequence of diets was reversed. **Results:** In group A, after one week of CML-rich diet we observed slightly elevated fasting serum CML levels (294 ± 9 vs. 329 ± 9 ng/ml, $p < 0.05$) and increased urinary CML excretion rates (1.3 ± 0.1 vs. 1.7 ± 0.2 mg/day, $p < 0.01$), while CML-poor diet decreased urinary CML excretion rates (1.2 ± 0.2 vs. 0.6 ± 0.2 mg/day, $p < 0.01$). Similar changes in serum and urine CML levels were observed in group B as well. CML-rich diet resulted in increased urinary albumin excretion in group A (8 ± 1 vs. 31 ± 12 mg/day, $p < 0.05$), while all other changes in albumin excretion were insignificant. Creatinine clearance and blood pressure remained unchanged during the study. **Conclusions:** Elementary CML-rich protein overload induces a slight rise in serum and urine CML levels as well as in albumin excretion rates indicating potential uptake and turnover of AGEs from heated food.

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EVALUATION OF GLYCATED INSULIN IN PLASMA AND BIOLOGICAL TISSUES OF DIABETIC ANIMAL MODELS

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Aims: To evaluate the glycation of insulin in plasma and biological tissues of diabetic animal models. **Methods:** Polyclonal antibodies of high specificity and sensitivity were produced against glycated insulin and used in RIA and immuno-cytochemistry (ICC) to assess glycated insulin in lean mice ($n=10$), ob/ob mice ($n=10$) and hydrocortisone (HC) treated diabetic rats ($n=10$; 80 mg/kg/day). **Results:** Glycated insulin circulated at 0.1 ± 0.04 ng/ml and 2.2 ± 0.1 ng/ml in lean and obese mice corresponding to 12.5 and 9.8% total insulin, respectively. The concentration of glycated insulin was elevated 22-fold ($P < 0.001$) in obese mice (glucose 22 ± 0.9 mmol/l) compared to controls (glucose 2.0 ± 0.4 mmol/l). In the pancreas, glycated insulin was 48 ± 10 and 83 ± 4 ng/g wt ($P < 0.05$) in lean and obese mice respectively, representing 2% total insulin (4.6 ± 0.17 μ g/g wt) in the diabetic pancreas. ICC revealed fluorescent positively stained cells in pancreatic islets from HC-treated diabetic rats (glucose 26 ± 1.0 mmol/l). Fasting of HC-treated rats, resulted in 3-fold and 15-fold reductions in plasma glycated insulin ($P < 0.01$; from 0.85 ± 0.16 ng/ml) and insulin ($P < 0.001$; from 9.25 ± 0.50 ng/ml) respectively. Following a 30 min feeding period in these insulin resistant rats, plasma concentrations of glucose, insulin and glycated insulin increased ($P < 0.001$) rapidly with 1.4-, 1.6- and 2.9-fold elevations, respectively. Injection of HC-treated rats with insulin (50 U/kg) resulted in a rapid 33% decrease of plasma glucose ($P < 0.001$) and a marked 4-fold increase in plasma insulin ($P < 0.01$), whereas glycated insulin concentrations remained unchanged. **Conclusions:** Glycation of cellular insulin appears to be a glucose concentration dependent process. The physiologically regulated secretion of glycated insulin into the circulation suggests a role in the pathogenesis of diabetes.

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IDENTIFICATION OF Tyr¹-GLUCITOL GIP IN INTESTINES OF DIABETIC MICE WITH ENHANCED INSULIN-RELEASING ACTIVITY
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Aims: To evaluate glycosylated GIP (Tyr¹-glucitol GIP) in intestinal extracts and compare its ability to stimulate cyclic AMP production and insulin secretion with native GIP. **Methods:** Small intestines from lean control and hyperphagic obese diabetic (ob/ob) mice (aged 20-25 weeks, n=10) were extracted in acid ethanol. Glycosylated and non-glycosylated GIP were separated by GlycoGel B affinity chromatography and quantified by fully cross-reacting (IR) GIP radioimmunoassay. Tyr¹-glucitol GIP was produced by incubating synthetic GIP under hyperglycaemic reducing conditions, purified by RP-HPLC and characterized using electrospray ionization mass spectrometry. Insulin release (mean \pm SEM, n=8) was measured in clonal pancreatic BRIN-BD11 cells following acute 20 min incubations. Intracellular cAMP production was measured (n=6) using Chinese hamster lung fibroblast (CHL) cells stably transfected with human GIP receptors. **Results:** Total IR-GIP detected in intestines of obese diabetic mice (451 ± 66 pmol/g) was significantly greater ($P < 0.05$) than in lean mice (251 ± 115 pmol/g) and Tyr¹-glucitol GIP represented 21% (90 ± 19 pmol/g) of total GIP extracted from obese mice but was below detection limits in lean controls. Native GIP dose-dependently stimulated insulin secretion ($P < 0.001$) by 1.2- to 1.8-fold over the concentration range 10^{-13} to 10^{-8} mol/l at 5.6 mmol/l glucose (basal 2.0 ± 0.1 ng/ 10^6 cells/20min). Tyr¹-glucitol GIP was 25% more potent at stimulating insulin secretion compared to native GIP ($P < 0.001$) at 10^{-8} mol/l. Upon binding to CHL cells, GIP and Tyr¹-glucitol GIP evoked a marked 1.2- to 1.8-fold stimulation of cAMP production. The calculated EC₅₀ values for these peptides were 18.2 and 2.03 nmol/l, respectively. **Conclusions:** N-terminally glycosylated GIP is present in the intestines of obese diabetic (ob/ob) mice and this structural modification increases cellular cAMP production and insulin secretion *in vitro*.

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DO SERUM LOW MOLECULAR WEIGHT ADVANCED GLYCATION END PRODUCTS CORRELATE WITH COMPLICATIONS ASSOCIATED WITH DIABETES?

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Background and Aims: Low molecular weight advanced glycation end products (AGEs) have been suggested to play a role in the initiation of complications associated with diabetes.

Materials and Methods: We have used a simple fluorescent assay (excitation wavelength 247nm, emission wavelength 440nm) to quantify these molecules in serum after precipitation with trichloroacetic acid. AGEs were quantified in arbitrary fluorescence units (AFU) due to the lack of any consensus standard for AGEs. We have explored the circulating levels of AGEs in an unselected diabetic clinic population of 443 patients (203F, 240M) mean age 57 ± 15.2 years with other correlates (HbA_{1c}, urinary albumin/creatinine ratio (ACR), and blood pressure) of diabetic control and complications. Retinopathy was present in 176 of the diabetic patients. Serum from 106 (60F, 46M) unselected non-diabetic subjects, mean age 52 ± 12.9 , acted as controls.

Results: Serum AGEs in the diabetic group (mean 13.6 AFU ± 36) were significantly higher ($p < 0.0001$) than in the control group (mean 6.2 AFU ± 2.9). There was no significant difference ($t = -0.83$, $p = 0.41$) between serum AGEs in the diabetic male (mean 15.0 AFU ± 44) and female (mean 12.1 AFU ± 22.8) groups. There was no correlation between serum AGEs and HbA_{1c} in the diabetics ($r = 0.07$, $p = 0.1259$). The strongest correlation of serum AGEs was with urinary ACR ($r = 0.23$, $p < 0.0001$). There was no significant difference in serum AGE levels between the diabetics with and without retinopathy ($p = 0.99$).

Conclusions: We have demonstrated that low molecular weight serum AGEs correlate better with indicators of macrovascular disease (urinary ACR) rather than microvascular disease in diabetic subjects.

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Hydrogen peroxide and pentosidine productions by myosin in diabetic myocardium.

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Aim: The purpose of this study was to examine myocardial H₂O₂ and pentosidine production from the Maillard reaction in NIDDM. **Methods:** A novel model using physiological levels of urea (a H₂O₂ stabilizer) was developed to monitor H₂O₂ production from myosin in long-term incubation with various concentrations of glucose *in vitro*. H₂O₂ was assayed by a xylenol assay. Pentosidine levels in myosin were determined by HPLC analysis. **Results:** Our *in vitro* experiments demonstrated that both H₂O₂ and pentosidine productions from the Maillard reaction of myosin were in a glucose-dose dependent manner. Molecular experiments showed that pentosidine formation was significantly enhanced by hydroxyl radical generated from H₂O₂ via Fenton reaction. Corresponding to the *in vitro* data, myocardial levels of H₂O₂ and pentosidine were higher in NIDDM group (mean \pm SD: control, n=10; NIDDM, n=7; H₂O₂, 0.10 ± 0.01 vs. 0.19 ± 0.02 (μ M/g); pentosidine, 2.02 ± 0.17 vs. 3.17 ± 0.21 (nM/mg), both $p < 0.05$). In addition, the levels inversely correlated with left ventricular ejection fraction (n=17, H₂O₂ vs. LVEF, $r = -0.76$; n=17, pentosidine vs. LVEF, $r = -0.73$; both $p < 0.05$). Immunostaining with an anti-pentosidine antibody showed fine pentosidine granules in association with distortion, disruption, and destruction of the striae in diabetic myocytes. **Conclusions:** Our data identified a novel source of myocardial H₂O₂ production in patients with NIDDM. The high levels of H₂O₂ and pentosidine may synergistically contribute to cardiac damage in NIDDM.

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INCREASE IN SERUM LEVELS OF ADVANCED GLYCATION ENDPRODUCTS IN HIGH RISK CORONARY ARTERY DISEASE PATIENTS IS MODIFIED BY THE ACE INHIBITOR RAMIPRIL

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Background and Aims: Advanced glycation endproducts (AGEs) which are formed by glycation or glycoxidation of proteins and lipids, are assumed to be participants in the premature atherosclerotic process taking place in diabetic patients. Human endothelial cells and macrophages possess receptors for AGE, and AGEs have been demonstrated in atherosclerotic plaques. Thus, it has been suggested that AGEs play a role in the atherosclerotic process in general, and not only in diabetes. Little is known about circulating AGEs in patients with coronary artery disease, and no study has examined the effect of medical intervention on circulating AGEs. ACE inhibitors have in experimental studies demonstrated beneficial molecular effects that might theoretically influence AGEs. Accordingly, we decided to investigate AGEs in high-risk coronary artery patients included in a clinical trial with ACE inhibitors. **Materials and Methods:** We applied our recently developed immunoassay for measurement of serum AGEs utilizing a polyclonal anti-AGE antibody and time delayed fluorescence (DELFA). Patients examined were those included at one centre in the Heart Outcomes Prevention Evaluation (HOPE) trial. All patients were randomised at baseline to ramipril (R) or placebo (P). Forty-three patients (36 men, 7 women, mean age of 66.5 ± 6.0 years) with coronary artery disease and 40 months follow-up were studied, those with diabetes or renal impairment were excluded. The R and P groups comprised 24 and 19 patients respectively. Fasting blood samples were collected and serum AGEs were measured both at baseline and after 40 months. **Results:** Serum levels of AGEs (median (5-95 percentile)) increased significantly from baseline to 40 months both for the R group, 5.8 (2.7-14.1) U/ml to 7.2 (2.4-21.0) U/ml, $p < 0.005$, and for the P group 4.6 (0.3-17.2) to 7.7 (4.3-23.2) U/ml, $p < 0.005$, respectively. The increase was significantly lower in the R group than in the P group ($p < 0.05$). **Conclusions:** Serum levels of AGEs increased in high-risk patients with coronary artery disease during the study period of 3.5 years. The increase was significantly lower in the ramipril group than in the control group. The ACE inhibitor ramipril, which in the HOPE study substantially reduced cardiovascular mortality and morbidity, might interfere with the accumulation of AGEs and thereby have a protective effect in the development of atherosclerosis.

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Cardiovascular Complications and Oxidative Stress

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ASCORBIC ACID SUPPLEMENTATION REDUCES BLOOD PRESSURE AND ARTERIAL STIFFNESS IN PATIENTS WITH TYPE 2 DIABETES

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Background and Aims: Oxidative stress is increased in diabetes. Free radicals can degrade endothelial derived nitric oxide (NO). Reduced NO bioavailability may cause increased blood pressure and vascular dysfunction. We examined the effects of ascorbic acid supplementation on blood pressure and artery stiffness in type 2 diabetes. **Materials and Methods:** 22 patients with uncomplicated type 2 diabetes (age 50-70, M/F 17/5) were recruited and randomised in a double blind manner to ascorbic acid 500mg daily or placebo for one month. Brachial systolic and diastolic blood pressure (BSBP, BDBP) were measured with the Omron HEM-705CP. The SphygmoCorTM Pulse Wave Analysis System was used to record radial artery pressure waveforms and generate the corresponding central aortic pressure waveforms. From these waveforms central aortic systolic and diastolic blood pressure (ASBP, ADBP), augmentation index (AgIx, measure of systemic arterial stiffness), the timing of wave reflection (Tr, measure of aortic pulse wave velocity) and the Buckberg Subendocardial Viability Ratio (SEVR, measure of endocardial perfusion) were derived.

Results:

	Placebo (n=11)		Ascorbic Acid (n=11)	
	Baseline	1 month	Baseline	1 month
BSBP (mmHg)	146.7±10.9	143.9±12.3	142.3±12.5	133.7±11.6**
BDBP (mmHg)	85.1±6.4	85.4±5.7	83.7±5.2	80.5±6.2*
ASBP (mmHg)	133.7±9.5	131.3±9.5	130.2±13.4	120.5±12.7***
ADBP (mmHg)	86.3±6.5	86.6±5.7	84.5±5.2	81.5±6.4
AgIx (%)	25.9±6.6	25.7±5.6	25.7±5.6	19.8±5.3***
Tr (ms)	140.0±6.6	139.5±4.5	137.3±13.9	144.6±9.6**
SEVR (%)	146.7±21.9	145.1±19.4	147.5±21.3	159.3±21.2*

Data = mean±SEM. *p<0.05, **p<0.01 versus baseline; †p<0.05 versus placebo.

Conclusions: Ascorbic acid lowered both peripheral and central aortic blood pressure, reduced arterial stiffness and improved endocardial perfusion. Ascorbic acid supplementation may therefore be of benefit to patients with type 2 diabetes.

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ERYTHROCYTE REFRACTIVE INDEX, MEMBRANE FLUIDITY AND PLASMA ANTIOXIDANT STATUS IN DIABETIC PATIENTS

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Background and Aims: It has been shown that diabetes is associated with functional and structural changes of erythrocytes. Our study was designed to clarify the relationship of glycated haemoglobin (HbA_{1c}) with erythrocyte refractive index, fluidity of erythrocyte membranes, and antioxidant status in plasma. **Materials and Methods:** 18 diabetic (NIDDM and IDDM) patients (D) before (HbA_{1c} >10%) and after 90 days of antidiabetic therapy (HbA_{1c} <6.5%) and 20 healthy controls (C). All patients were without diabetic complications, peripheral vascular disease, hypertension and dyslipidemia. The groups were matched for age, sex and body mass index. Erythrocyte membrane fluidity was determined by means of hydrophobic fluorescence probe 1,6-diphenylhexa-1,3,5-triene. Erythrocyte refractive index was evaluated by polarising-interference microscopy at different pH. Antioxidant status in plasma - by measuring radical cation 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) scavenging activity.

Results: Before therapy diabetic patients had higher values (mean±SD; Mann-Whitney U test) of fluorescence anisotropy (FA), erythrocyte refractive index (RI) and plasma antioxidant status (AS) than controls (FA: D 0.233±0.010 vs C 0.208±0.002, p<0.0001; RI: D 1.61±0.02 vs C 1.14±0.33, p<0.0001, pH=6.9 and AS: D 47.6±2.9 vs C 38.6±2.7%, p<0.0001). After therapy no significant differences were found. HbA_{1c} correlated with FA (r = 0.62; p<0.05), RI (r = 0.56; p<0.05) and AS (r = 0.59; p<0.05). **Conclusions:** Poor diabetes control causes significant changes of erythrocyte membrane fluidity, erythrocyte refractive index and plasma antioxidant status. Abnormal properties of erythrocytes may be the result of non-enzymatic glycation of proteins and overproductions of free radicals.

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Effect of probucol treatment on malondialdehyde-modified low-density lipoproteins in type 2 diabetic patients with hypercholesterolemia.

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Background and Aims: Numerous studies indicated that oxidized low-density lipoproteins (ox-LDL) plays a critical role in the pathogenesis of atherosclerosis. Probucol, 4,4'-(isopropylidenedithio) bis(2,6-di-tertbutylphenol), is a widely used cholesterol lowering drug. This drug is accepted to have antioxidant actions, however, the clinical effect of probucol on lowering ox-LDL is still unknown. We studied the effect of probucol on malondialdehyde-modified LDL (MDA-LDL) as ox-LDL marker and other lipids markers in type 2 diabetic patients with hypercholesterolemia.

Materials and Methods: All data were shown as mean±SE. Subjects were thirty type 2 diabetic patients (male:4, female:26, age: 58.6±1.9 yr. duration of diabetes; 7.2±0.8 yr; incidence of macroangiopathy; 23.3 %) with hypercholesterolemia (T.Chol >220 mg/dl). All patients gave their informed consent to this study. Before and 3 months after probucol (500mg/day) treatment, MDA-LDL (measured by ELISA with ML25 antibody) and other lipids markers [T.Chol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), lipid peroxidation (LPO), lipoprotein a (Lp(a)), remnant-like particle cholesterol (RLP-C)] were measured.

Results: At the start of treatment, MDA-LDL (127.5±5.9 U/L) was positively correlated with LPO (5.4±0.4 nmol/ml), TC (242.8±5.1 mg/dl), LDL-C (149.9±14.4 mg/dl), and HbA_{1c} (7.5±0.2 %) (P<0.05). Three months-treatment of probucol significantly reduced MDA-LDL (108.5±6.1 U/L), LPO (4.3±0.4 nmol/ml), TC (222.3±3.7 mg/dl), HDL-C (40.8±1.7 mg/dl), and LDL-C (142.7±5.7 mg/dl) (P<0.05), although the other markers were not changed. Furthermore, % change in MDA-LDL by probucol treatment also positively correlated with % changes in LPO (P<0.05) and HbA_{1c} (P<0.001). There was no side effects observed by the treatment.

Conclusions: These data firstly demonstrate that probucol clinically reduced MDA-LDL. Probucol may be useful as a drug to prevent atherosclerosis in type 2 diabetic patients by quantitatively and qualitatively improving dyslipidemia.

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EFFECTS OF α-LIPOIC ACID ON LIPID AND PROTEIN OXIDATIVE DAMAGE AND LYSOSOMAL ENZYME ACTIVITY IN TYPE 1 DIABETES

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Background and Aims: The increased free radical production in diabetes mellitus (DM) is believed to induce the lipid and protein oxidative modifications and may be responsible for labilization of lysosomal membrane. In this randomized, double-blind study we investigated the effects of antioxidant α-lipoic acid (ALA) on oxidative damage of lipids and proteins and serum activity of lysosomal enzymes (LEs) in DM. **Materials and Methods:** 23 type 1 DM patients with symptomatic peripheral neuropathy (11 M/12 F; age 30.1±5.5 yrs; HbA_{1c} 11.0±0.5%) were randomly assigned to treatment with a daily intravenous infusions of 600 mg ALA (12 patients) or placebo (11 patients) for 3 weeks. 25 healthy subjects acted as controls. The content of malondialdehyde (MDA), carbonyl groups in proteins and activity of LEs: acid RNA-se and N-acetyl-glucosaminidase (NAG) were measured in blood serum. **Results:** Mean blood glucose and HbA_{1c} levels did not differ between the groups during the study. At baseline the level of MDA, protein carbonyls, acid RNA-se and NAG activity were increased significantly in both diabetic groups respect to control (all p<0.05). Therapy with ALA reduced MDA (1.76±0.57 μmol/ml at baseline vs 1.1±0.27 after the treatment), activity of acid RNA-se (7.9±0.9 vs 6.9±0.8 nmol·h⁻¹·ml⁻¹) and NAG (802±91 vs 713±92 nmol·h⁻¹·ml⁻¹) in serum (all p<0.05). There was a trend toward a decreasing in protein carbonyls in ALA group (888±141 vs 853±83 nmol/mg, p>0.05). In placebo group parameters did not change significantly (MDA: 1.68±0.62 vs 1.57±0.16 μmol/ml; acid RNA-se: 7.8±1.0 vs 7.7±1.2 nmol·h⁻¹·ml⁻¹; NAG: 827±89 vs 812±94 nmol·h⁻¹·ml⁻¹; protein carbonyls: 863±136 vs 869±66 nmol/mg; all p>0.05). **Conclusions:** The results demonstrate that α-lipoic acid administration may reduce oxidative damage of serum lipids and proteins in patients with type 1 DM. Moreover, α-lipoic acid decrease high serum activity of LEs, an effect that may be due to the inhibition of lysosomal membrane labilization mediated by oxidative stress.

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Inhibition of high dose glucose-induced overproduction of superoxide anion in J-774 cells by various HMG-CoA inhibitors.

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Background and Aims: It is suggested that oxidant stress causes microangiopathy and arteriosclerosis in diabetic patients. The origin of oxidants (oxygen-derived free radicals) is not still completely known, however, overproduction of oxygen-derived free radicals (e.g. superoxide anion: O₂⁻) from macrophage is now thought to cause them. On the other hand, attention is called to the fact that HMG-CoA inhibitors (statins) reduce neointimal inflammation of arteriosclerosis. Because it is not clear that statins affect the overproduction of O₂⁻ by macrophages under high dose glucose condition, we studied the effect of various statins on the generation of O₂⁻ by J-774 macrophage-like cell line under high dose glucose culture.

Materials and Methods: The basal and stimulated generations of O₂⁻ were measured by chemiluminescence amplified with a Cypridina luciferin analog. The stimulated generation of O₂⁻ was assessed with the maximally changed value of chemiluminescence in response to 100 nM phorbol 12-myristate 13-acetate (CLA-DCL). J-774 cells were cultured with medium (DMEM) containing 100-600 mg/dl of glucose, with or without various statins (cerivastatin, fluvastatin, and nisvastatin). The effects of co-incubation with statins and mevalonic acid, and a NADPH oxidase inhibitor (diphenyleneiodonium chloride: DPI), and a protein kinase C inhibitor (GF-109803X: GF) were also studied.

Results: Three-days culture with high dose glucose apparently increased both basal chemiluminescence and CLA-DCL in a dose dependent manner (600 mg/dl glucose; basal CL:181%, CLA-DCL:202%, P<0.01). The high dose glucose-induced increases in both basal CL and CLA-DCL were also time-dependent (7 days with 600 mg/dl glucose; basal:346%, CLA-DCL:278%, P<0.05). Every statins (0.1-10 µM) significantly inhibited the high dose glucose-induced overproduction of O₂⁻ in a dose dependent manner (P<0.001), and the statins-induced inhibition was clearly prevented by co-incubation with 100 µM mevalonic acid (P<0.05). Both 20 µM DPI and 2 µM GF also inhibited the high dose glucose-induced increase in O₂⁻ (P<0.001).

Conclusions: These data suggest that high dose glucose causes overproduction of O₂⁻ generated through NADPH oxidase and protein kinase C pathways, and statins suppress the overproduction of O₂⁻ in J-774. Statins may be useful as a drug to prevent microangiopathy and arteriosclerosis by inhibiting oxidant stress in poorly controlled diabetic patients.

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Fenofibrate inhibited high dose glucose-induced overproduction of superoxide anion by human monocytes.

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Background and Aims: Oxidative stress (e.g. overproduction of superoxide anion) is known to cause microangiopathy and arteriosclerosis in diabetes. Monocyte is one of the cells that produce superoxide anion and it may be possible that overproduction of oxygen-derived free radicals (e.g. superoxide anion) from monocytes causes microangiopathy and arteriosclerosis. On the other hand, it is recently appeared that peroxisome proliferator-activated receptor [alpha] (PPAR [alpha]) modulates inflammation, and fibrates, antilipidemic agents, exert their pharmacological effects via PPAR [alpha]. To evaluate whether fenofibrate, one of fibrates, affect oxidative stress in diabetes, we studied the in vitro effect of fenofibrate on the generation of superoxide anion in normal monocyte under high-dose glucose condition. **Materials**

and Methods: The generation of superoxide anion was measured by chemiluminescence amplified with a cypridina luciferin analog. The stimulated generation of superoxide anion was assessed with the maximally changed value of chemiluminescence in response to 100nM f-MLP (CLA-DCL). Monocytes were separated from blood of normal healthy male subjects and cultured with medium (DMEM) containing different dosages (100-600 mg/dl) of glucose for 3 days, with or without fenofibrate, or a NADPH oxidase inhibitor (diphenyleneiodonium chloride: DPI), or a protein kinase C inhibitor (GF-109203X: GF). Effect of Wy-14643, one of the PPAR[alpha] agonist, was also studied. **Results:** High-dose glucose significantly increased CLA-DCL in a dose dependent manner (P<0.05). 600 mg/dl glucose-induced increase in CLA-DCL was apparently inhibited by either 20µM DPI or 2µM GF (P<0.01). Fenofibrate (10µM-1mM) also significantly inhibited the high glucose-induced increase in CLA-DCL in a dose dependent manner (P<0.01), and 50µM Wy-14643 also inhibited the increase in CLA-DCL (P<0.01). **Conclusions:** these data provide the first evidence that in human monocytes high-dose glucose causes the overproduction of superoxide anion through NADPH oxidase and protein kinase C systems, and fenofibrate decreases the overproduction of superoxide anion via PPAR[alpha]. Fenofibrate may be useful as a drug to prevent microangiopathy and arteriosclerosis by inhibiting oxidative stress in poorly controlled diabetic patients.

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Baseline diene conjugates in plasma low-density lipoprotein and paraoxonase activity in Type-2 diabetes and controls

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Background and Aims: Oxidative modification of low-density lipoproteins (LDL) in plasma and in the arterial wall may promote the development of atherosclerosis. Surrogate measurements for LDL oxidation/oxidisability include measuring the susceptibility of LDL to copper-induced oxidation *ex vivo*. This method measures the copper-induced production of LDL conjugated dienes (mostly derived from modified linoleic acid esters) by absorbance at 234 nm. More recently, a well validated method that measures diene conjugates LDL at baseline without copper stimulation has been developed, and appears to be a more accurate reflection of the presence of oxidatively modified plasma LDL *in vivo*. We measured plasma LDL baseline diene conjugates in Type-2 diabetes and controls as an index of *in vivo* LDL modification. The relationships between activity of the HDL-associated antioxidant enzyme paraoxonase and LDL baseline diene conjugation were also examined.

Materials and Methods: We studied 57 patients with Type-2 diabetes without vascular disease (mean age 58.1 ± 10.1 years; mean HbA1c 8.01 ± 0.98 %), and 48 age- and sex-matched controls. The level of plasma LDL baseline diene conjugates was measured using a citrate-heparin precipitation method, and plasma paraoxonase activity was assessed spectrophotometrically by measuring the rate of p-nitrophenol produced at 405nm.

Results: Mean LDL baseline diene conjugates were significantly higher in the Type-2 patients than in controls (25.5 ± 15.3 vs 19.7 ± 9.4 µmol/l; p = 0.025), but there was no significant difference in LDL concentrations between either gender or group (p > 0.05). Paraoxonase activity did not differ significantly between groups (p > 0.05), and was unrelated to LDL baseline diene conjugate concentrations (p > 0.05).

Conclusions: Measures of LDL baseline diene conjugation may be a better index of *in vivo* oxidative LDL modification than the usual surrogate methods. LDL baseline diene conjugate concentrations were significantly higher in the Type-2 diabetes group indicating increased *in vivo* LDL oxidative modification, and paraoxonase had no detectable protective effect against LDL oxidation.

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SPECIFIC GLUCOSE ENHANCEMENT OF HEMIN-(Fe³⁺)-CATALYZED LDL OXIDATION IN VITRO AND IN VIVO

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Background and Aims: Clinical and experimental evidence suggests that oxLDL is increased in diabetes. The mechanisms for these observations remain unclear. We hypothesized that redox-active glucose *per se* is involved in LDL oxidation. **Materials and Methods:** We examined the effect of pathophysiological concentrations of glucose (6.5 to 25 mM) on hemin-(Fe³⁺)-catalyzed oxidation of LDL apoB-100 *in vitro*. Oxidative modification of apoB-100 has been determined by relative electrophoretic mobility (REM) and formation of 5-hydroxy-2-aminovaleric acid (HAVA). HAVA is highly specific for hemin-(Fe³⁺)-catalyzed oxidation of apoB-100 proline and arginine residues. Furthermore, HAVA levels have been determined in subjects with impaired glucose tolerance (IGT, n=12; glucose, 6.48 ± 0.82 mmol/L), type 2-diabetics (DM, n=10; 7.34 ± 0.71 mmol/L) and controls (n=10; 4.52 ± 0.64 mmol/L). **Results:** *In vitro*, oxidation of LDL was stimulated by glucose resulting in 2- to 5-fold greater REM (p<0.01) and 1.2- to 2-fold higher HAVA levels (p<0.01) than control incubations without glucose. The effect of glucose was inhibited by the redox-inert iron-chelating agent 1,2-Dimethyl-3-hydroxypyrid-4-one and superoxide dismutase, resp. Thus, enhancement of LDL oxidation by glucose *in vitro* seems to be specific for hemin-(Fe³⁺)-catalyzed oxidation. *In vivo*, LDL HAVA levels were significantly higher in subjects with IGT (0.052±0.013 mol/mol apoB-100; p<0.01) and DM (0.064±0.011 mol/mol apoB-100; p<0.01), resp., when compared with controls (0.011±0.004 mol/mol apoB-100). However, a weak correlation between LDL HAVA and fasting glucose levels has been found only in DM (Kendall correlation analysis; r=.533; p<0.01). **Conclusion:** These results provide one potential mechanism for enhanced LDL oxidation in diabetes.

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DIABETES-RELATED CHANGES CAN LEAD TO INCREASED IRON-INDUCED LIPID PEROXIDATION

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Background and Aims: One of the pathogenic mechanisms postulated to modulate the susceptibility of diabetic patients to the adverse effects of hyperglycaemia is oxidative stress. High glucose increases release of free radicals and impairs antioxidant synthesis and recycling. Less is known on its effects on one of the major extracellular antioxidant mechanisms, namely the sequestration of transition metals in forms incapable of stimulating free radical reactions. We aimed therefore to study the influence of glucose and glycation on the capacity of transferrin to inhibit iron-induced lipid peroxidation. **Methods:** An in vitro liposome model containing different concentrations of iron and ascorbate distinguished between iron-binding (IB), iron-oxidising (IO) and chain breaking (CB) antioxidant capacities which were expressed as % inhibition of the production of thiobarbituric acid reactive substances (TBARS) when compared to the reaction mixture without antioxidant. **Results:** Human apotransferrin (Tf) showed no IO or CB capacity but inhibited peroxidation by IB in a dose dependent manner: 46±3% inhibition at 0.5 mg/mL, 86±4% at 2 mg/mL and 88±6% at 5 mg/mL (M±SD n=12), p <0.0001 when compared to control without antioxidant. Addition of D-glucose 5.6 and 33.3 mmol/L to liposomes caused 19% (p0.0001) and 35% (p<0.0001) increases in TBARS resp. but did not enhance the effects of iron and ascorbate significantly. Incubation of Tf with D-glucose for 10, 14 or 21 days at 37°C which resulted in time-dependent increases in glycation: 4.8, 8.6, 32.3 and 327 µmol fructosamine/g protein at 0, 5.6, 33.3 and 1000 mmol/L glucose respectively, caused no changes in IO capacity but significant decreases in IB from 89±0.5 % after 0 mmol/L to 85±2% after 33.3 mmol/L and 0.8±4.5% after 1000 mmol/L glucose, p<0.001. Unexpectedly Tf which had been glycated by incubation with 1000 mmol/L glucose stimulated lipid peroxidation in the CB condition by 10±8%, p<0.01 vs incubation with 0 mmol/L glucose. **Conclusion:** These results suggest that lowered serum transferrin, its glycation and the presence of high glucose can, by enhancing the pro-oxidant effects of iron, contribute to the increased lipid peroxidation observed in diabetes mellitus. The molecular mechanisms behind these observations need further investigation.

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Transition metal catalysed free radical production and defective endothelium-dependent relaxation in the mesenteric vasculature of diabetic rats.

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Background and Aims: Diabetes causes endothelial dysfunction; the involvement of oxygen free radicals in impaired nitric oxide (NO) mediated vasodilation has been well documented. However, the processes whereby free radicals are generated remain to be fully elucidated. Transition metal catalysed hydroxyl radical production is a potentially important source. In the arterioles that control tissue blood flow, other local vasodilators such as endothelium-derived hyperpolarizing factor (EDHF) have an important role. It is not known whether this mechanism is susceptible to elevated oxidative stress in diabetes. The aim was to assess whether an antioxidant treatment strategy using the transition metal chelator, trientine, could prevent the development of vascular EDHF and NO deficits in experimental diabetes. **Materials and Methods:** After 4 weeks of streptozotocin-induced diabetes in rats, the responses of the isolated, perfused mesenteric vascular bed to vasoactive drugs were examined. Treated diabetic rats were given trientine in the drinking water such that the daily dose was approximately 20 mg/kg. **Results:** Acetylcholine (ACh) induced maximal relaxation of the phenylephrine-precontracted mesenteric bed was 31.5% reduced from 91.8 ± 2.9% (± SEM) to 62.9 ± 3.6% by 4 weeks of diabetes (p<0.001). Trientine treatment provided 75.9% protection (p<0.001) such that maximal relaxation (84.8 ± 1.9%) was not significantly different from that of the nondiabetic control group. Pre-incubation with NG-nitro-L-arginine eliminated the NO component of vasodilation to reveal responses mediated by other vasodilators. Under these conditions, maximum relaxation to ACh was reduced to 59.5 ± 5.0% in controls. There was a 42% diabetic deficit (34.4 ± 2.2%; p<0.001), which was 77.3% attenuated (p<0.01) by trientine treatment, maximum relaxation (53.8 ± 2.7%) being in the nondiabetic range. These responses were not significantly altered by co-incubation with the cyclooxygenase inhibitor, flurbiprofen, indicating that they were mediated by EDHF. Neither diabetes nor trientine treatment altered endothelium-independent relaxation to the NO donor, sodium nitroprusside, in the presence or absence of NG-nitro-L-arginine. **Conclusions:** The data show that experimental diabetes has deleterious effects on NO and EDHF-mediated vasodilation and suggest that transition metal catalysed oxygen free radical production is an important determinant of impaired endothelium function. Given the relative importance of EDHF in microcirculatory control, this mechanism may make a major contribution to the aetiology of diabetic microangiopathic complications.

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Effect of oxidized LDL on the amount of insulin receptor and Gi alpha proteins in the caveolae of bovine aortic endothelial cells (BAEC).

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Background and Aims: Oxidized LDL(ox-LDL) may induce endothelial cell dysfunction which results in atherosclerosis or insulin resistance. Several studies have shown that ox-LDL inhibits signaling pathways mediated by inhibitory GTP-binding proteins(Gi-proteins). And G-protein coupled receptors(GPCRs) can be internalized via caveolae. Caveolae are small flask-shaped invaginations of the plasma membrane, characterized by high levels of cholesterol and glycosphingolipids and also by the presence of caveolin, a 20-24kDa integral membrane protein. G-proteins are enriched within caveolae membranes, where caveolin-1 directly interacts with the alpha subunits of G-proteins. It is reported that functional changes of G-proteins such as mutational or pharmacological activation of G-proteins affect direct interaction between G-proteins and caveolin-1. Hereby we investigated the effect of ox-LDL on the change of the amount of insulin receptor and Gi alpha proteins in the caveolae.

Materials and Methods: Ox-LDL was prepared by exposing samples of native LDL to CuSO₄(5µM) for 24 hours. We treated BAECs with increasing concentrations of ox-LDL(0-100ug/ml) for various duration.

Results: Treatment of ox-LDL on the BAEC results in decrease of insulin receptor, Gi alpha2 and Gi alpha3 proteins in caveolae(insulin receptor : 66 %, Gi alpha2 protein : 33 %, Gi alpha3 protein : 66 %, p<0.05).

Conclusions: These results suggest that the change of the amount of insulin receptor and Gi-proteins in caveolae may induce endothelial cell dysfunction.

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PHENSUCCINAL IMPROVES LIPID PROFILE AND ENHANCES PARAOXONASE ACTIVITY IN DIABETIC RABBITS

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Background and Aims: Increased risk of cardiovascular disease in diabetic patients may be partially related to abnormal lipid metabolism and oxidative damage. The new succinate derivative Phensuccinal (Ph) is a potent antioxidant, which is currently in clinical trials for the prevention of diabetic nephropathy. The aim of the study was to evaluate the impact of long-term treatment with Ph on the lipid profile and activity of HDL-associated antioxidant enzyme paraoxonase (PON) in diabetic rabbits. **Materials and methods:** Male chinchilla rabbits were made diabetic by i.v. injection of diethylenetriamine (35mg/kg b.w.). Control rabbits (C) were given vehicle alone. In a week after diabetes induction animals were randomised into two groups: one group acted as diabetic control (D) and other group received Ph (50 mg/kg per os) for 3 months. At the end of the study blood was sampled in a fasting state for analysis of glucose, plasma insulin, serum total cholesterol (TC), LDL-C, HDL-C, triglycerides (TG), NEFA and lipid hydroperoxides (LHP). PON activity was measured in serum using paraoxon as a substrate. **Results:** Administration of Ph decreased basal hyperglycaemia (7.7±0.2 vs D: 15.6±1.7; C: 4.2±0.2 mmol/l, p<0.01) and increased plasma insulin (p<0.02) in comparison with diabetic controls. The treatment with Ph elevated HDL-C by 49 %, diminished LDL-C by 28 % and TG by 27 % compared to D-group (all p<0.02). Ph provided also reduction in TC by 15 % (p<0.05) and NEFA by 45 % (p<0.02) in comparison with diabetic controls. In addition, the use of Ph was associated with decrease in LHP levels (p<0.02 vs D-group) and enhancement of PON activity (59.0±1.1 vs D: 37.6±2.2; C: 81.0±2.1 U/l, p<0.01). **Conclusions:** These findings suggest that Ph exerts antiatherogenic effect due to improvement of glycaemic control, lipid profile and activation of antioxidant defence in diabetic rabbits. Thus the use of Ph may have implications in prevention of diabetic macrovascular complications.

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STIMULATION OF BLOOD FLOW BY C-PEPTIDE IN PATIENTS WITH TYPE 1 DIABETES

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Background and Aims: Proinsulin C-peptide binds to cell membranes, activates a G-protein coupled membrane receptor and Ca^{2+} -dependent intracellular signal transduction, resulting in activation of eNOS in endothelial cells. Infusion of C-peptide to type 1 diabetes patients increases blood flow to the kidneys, the skin and exercising muscle. This study is intended to examine the effect of C-peptide on forearm blood flow (FBF) and its concentration dependency.

Material and Methods: In a double blind randomized study we measured FBF, using venous occlusion plethysmography, during i.v.-infusion of recombinant human C-peptide or placebo at three different rates (1, 5 and 25 pmol/kg/min) for 60 min each in 10 type 1 diabetes patients. The patients were studied on two different occasions.

Results: Basal FBF was 22.0 ± 2.0 ml/min/L and increased significantly during C-peptide infusion (ANOVA $P < 0.001$). During infusion at the lower rate C-peptide concentrations rose to 0.3 ± 0.1 nM and FBF increased by $15 \pm 3\%$ above basal ($P < 0.001$). When the C-peptide concentration rose to 1.1 ± 0.1 nM during the intermediate rate infusion FBF increased further to $25 \pm 6\%$ above basal ($P < 0.005$). FBF did not show a significant further increase during the high rate infusion, despite C-peptide concentrations of 7.6 ± 0.3 nM. Thus, FBF rose in response to C-peptide in a concentration-dependent manner in the interval 0-1 nM but not at higher concentrations. The results are consistent with the previously reported C-peptide binding characteristics for isolated cells, indicating saturation of binding already at approximately 0.9 nM. It is concluded that in type 1 diabetes patients, C-peptide stimulates FBF in a concentration-dependent manner in the low nanomolar concentration range.

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Folate treatment improves endothelial function in hypertensive and insulin resistant patients.

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Background and Aims: Endothelial dysfunction and high homocysteine levels are correlated with cardiovascular disease and essential hypertension. In particular, high homocysteine levels are associated with impaired peripheral endothelium-dependent vasodilation. Several studies suggest that folic acid or B vitamins supplementations are able to decrease homocysteine levels and possibly to reduce cardiovascular risk. A link between endothelial dysfunction and the metabolic alterations typical of the Insulin Resistance Syndrome (IRS) has been previously demonstrated. However, little is known about the ability of folic acid plus vitamin B12 supplementation to improve both endothelial function and metabolic features of IRS. To this aim, we studied 16 patients (age 66.5 ± 1.9 yrs; BMI 27.6 ± 0.7 kg/m²) with essential hypertension, impaired fasting glucose according to ADA classification, hyperinsulinaemia, hypertriglyceridaemia.

Materials and Methods: A double blind randomized one month cross-over study with folic acid (5 mg/day) plus vitamin B12 (500 µg/day) or placebo was performed. Basal, post-ischaemic and post-nitrate forearm blood flow were evaluated by venous occlusion plethysmography and blood samples were withdrawn to measure metabolic parameters.

Results: Compared to placebo, folic acid plus vitamin B12 treatment significantly decreased homocysteine levels (10.9 ± 0.8 vs 17.2 ± 2.0 µmol/L; $p < 0.01$), mean blood pressure (104 ± 2 vs 111 ± 3 mmHg; $p < 0.05$) and forearm basal (37.9 ± 2.4 vs 49.2 ± 4.9 U; $p < 0.05$) and post-ischaemic vascular resistance (28.8 ± 2.8 vs 37.1 ± 2.7 U; $p < 0.05$) while a significant increment in post-ischaemic forearm blood flow (4.00 ± 0.28 vs 3.16 ± 0.17 ml/100 ml/min; $p < 0.02$) was observed. On the contrary, no differences were found on endothelium-independent nitrate-mediated vasodilation. Interestingly, no changes in blood glucose (108 ± 4 vs 111 ± 5 mg/dl, NS), insulin (18.2 ± 2.7 vs 19.0 ± 2.3 µU/ml; NS), HOMA index (5.0 ± 0.8 vs 5.3 ± 0.7 ; NS) and triglyceride levels (215 ± 25 vs 243 ± 32 mg/dl; NS) were seen.

Conclusions: Folic acid plus vitamin B12 supplementations were able to improve endothelium-dependent vasodilation independently from modification of the main features of IRS in these patients at high risk to develop cardiovascular disease.

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Increased pulse wave velocity in healthy offspring of patients with type 2 diabetes

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Background and Aims: Pulse wave velocity (PWV) is a simple non-invasive measurement of large artery structure and function. It is determined, in part, by arterial stiffness and vascular endothelial function. It increases with age and has been shown to correlate with cardiovascular risk. Healthy offspring of patients with type 2 diabetes have been shown to exhibit several features of the metabolic syndrome including insulin resistance and endothelial dysfunction. The aim of this study was to test the hypothesis that PWV would be increased in a group of offspring of diabetic patients (offspring) compared with a well-matched control group with no family history of type 2 diabetes (controls).

Materials and Methods: Offspring ($n=20$ {M=8, F=12}) were recruited via contact with patients attending the Diabetes Clinic. Controls ($n=20$ {M=8, F=12}) were recruited from hospital staff. Carotid-radial PWV was measured using COMPLIOR (Colson, France). Analysis of data was performed by an investigator blinded to subject status. Blood pressure and heart rate were measured in triplicate and blood was taken for measurement of metabolic and endothelial parameters. All measurements were made in a fasting, resting and supine state.

Results: Offspring and controls were well matched (mean{SD}) for age (33.1 { 9.6 } v 32.8 { 9.5 } yrs), body mass index (24.8 { 4.9 } v 24.3 { 3.4 } kg/m²), waist circumference (78.3 { 2.3 } v 76.3 { 2.5 } cm), systolic blood pressure (120 { 9.3 } v 119 { 14.2 } mmHg), pulse pressure (52 { 10.5 } v 53.5 { 9.3 } mmHg) and resting heart rate (71 { 8.7 } v 69 { 14.0 } b/min). Pulse wave velocity was significantly higher in the offspring (9.94 { 1.3 } m/sec) compared with controls (9.01 { 1.2 } m/sec) ($p=0.02$).

Conclusions: Carotid-radial PWV was 10% higher in healthy offspring of patients with type 2 diabetes compared with a control group which was tightly matched for sex, age, body mass index, blood pressure and heart rate. This suggests that vascular dysfunction, possibly in the form of increased arterial stiffness and endothelial dysfunction, is present from an early stage in subjects at higher risk of developing the metabolic syndrome. PWV may be a simple non-invasive measurement of vascular function in the early assessment of subjects at risk for developing metabolic and cardiovascular disease.

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INCREASE IN FLOW MEDIATED DILATATION BY CERVASTATIN

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Background and aims: Flow-mediated dilatation (FMD) is reduced in type 2 diabetes. Statin treatment has a remarkable effect on cardiovascular morbidity and mortality in diabetic patients with ischemic heart disease. The aim of the present study was to investigate the effect of cerivastatin on flow-mediated dilatation in type 2 diabetic patients with average lipid levels. **Materials and Methods:** 24 type 2 diabetic patients without ischemic heart disease and with average levels of blood lipids were investigated in a randomised, cross-over, controlled open-labeled study. FMD of the right brachial artery was examined using a high-frequency ultrasound device (Siemens Sonoline ®, 10 MHz) along with 5 min ischemia and nitroglycerin as control. Measurements were performed off-line by a blinded observer. After baseline measurements the patients were treated with cerivastatin or continued standard treatment. Three months later the patients switched treatment according to the cross-over design. **Results:** FMD was reduced at baseline compared to an age- and sexmatched control group (3.7 { 0.7 }% vs. 13.5 { 1.2 }%) (mean, SEM). FMD was 6.7 { 1.2 }% after cerivastatin compared to 2.1 { 0.6 }% after standard treatment ($p < 0.001$). The response to nitroglycerin was not changed by cerivastatin (13.2 { 1.2 }% vs. 13.2 { 0.9 }%). Total cholesterol was 4.0 { 0.14 } mmol/l after cerivastatin and 4.8 { 0.17 } mmol/l after standard treatment ($p < 0.000001$) while HDL-cholesterol did not change (1.05 { 0.07 } mmol/l vs. 1.07 { 0.06 } mmol/l). **Conclusions:** cerivastatin partially restores vascular wall function measured as flow mediated dilatation. This seems not to be explained by an effect at the smooth muscle cell level but is best explained by an improvement in endothelial function.

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OBES WOMEN ARE INSULIN RESISTANT BUT HAVE NORMAL ENDOTHELIAL FUNCTION

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Background and aims: Obesity or the associated insulin resistance increases the risk of developing type 2 diabetes and its vascular complications. Endothelial dysfunction has been suggested to precede, co-occur or be a consequence of insulin resistance. We performed invasive measurements of in vivo endothelial function and insulin sensitivity in a large group of obese (BMI 28-35 kg/m²) women and non-obese women.

Material and methods: 51 obese (age 38±1 yrs., BMI 32±1 kg/m²) women and 25 non-obese women (age 41±3 yrs., BMI 23±1 kg/m²) were studied. Endothelial function was assessed from forearm vasodilatory responses to intra-arterial infusions of the endothelium-dependent vasodilator acetylcholine, ACh (7.5 and 15 µg/min) and the endothelium-independent vasodilator sodium nitroprusside, SNP (3 and 10 µg/min). Insulin sensitivity was measured using the euglycemic insulin clamp technique (rate of insulin infusion 1.0 mU/kg·min).

Results: The obese women had higher diastolic blood pressure (82±1 mmHg vs. 74±2 mmHg, obese vs. non-obese, $p<0.0001$), serum triglycerides (1.5±0.1 mmol/l vs. 1.1±0.1 mmol/l, $p<0.05$) but lower HDL cholesterol (1.2±0.1 mmol/l vs. 1.7±0.1 mmol/l, $p<0.0001$) than the non-obese women. The obese were clearly more insulin resistant than the lean ones: fasting insulin (10±1 mU/l vs. 5±1 mU/l $p<0.0001$), fasting glucose (102±1 vs. 95±1, $p<0.01$) were higher in the obese women and whole body glucose uptake was markedly reduced (2.5±0.1 mg/kg·min vs. 5.7±0.4 mg/kg·min, $p<0.0001$ for obese vs. lean respectively). Vasodilatory responses to low (9.6±0.6 vs 10.0±1.0 ml/dl·min, obese vs. non-obese, NS) and high (12.3±0.7 vs 11.9±1.0 ml/dl·min, NS) doses of ACh and to low (8.5±0.3 vs 8.5±0.5 ml/dl·min, NS) and high (11.8±0.5 vs. 12.0±1.0 ml/dl·min, NS) doses of SNP were identical between the groups. **Conclusions:** This large group of obese women had normal endothelial function despite insulin resistance. These data suggest that insulin resistance precedes endothelial dysfunction rather than vice versa in obesity.

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ASSESSMENT OF 8-iso PGF₂α INVOLVEMENT IN THE ENDOTHELIAL DYSFUNCTION ASSOCIATED WITH TYPE 1 DIABETES

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Background and Aims: Oxidative stress is involved in the vascular endothelial dysfunction associated with diabetes mellitus. The isoprostanes are prostaglandin-like compounds formed as products of free radical-catalysed lipid peroxidation, and are now emerging as potential markers of oxidative stress in human vasculature. One such isoprostane, 8-iso PGF₂α, has recently been shown to be a potent vasoconstrictor in a range of vascular tissues. We have previously demonstrated that urinary levels of 8-iso PGF₂α in diabetic rats (streptozotocin, STZ-induced) are increased three fold, when compared with non-diabetic controls. The aim of the present study was to characterise effects of estimated plasma 8-iso PGF₂α concentrations (0.3 uM) corresponding to the elevated levels in diabetic urine (3 uM), on vascular relaxation in non-diabetic and diabetic rat aorta. **Materials and Methods:** Rats were given a single i.p. dose of STZ (60 mg/kg) and hyperglycaemia confirmed by measurement of blood glucose levels. Endothelium-dependent relaxations (EDR) of isolated aortic rings (mediated by acetylcholine, ACh) were measured in tissues pre-contracted with phenylephrine (PE) and maximal percentage relaxations (Rmax), relative to 30 uM ACh, were recorded. In separate experiments, contractions to 8-iso PGF₂α were measured. **Results:** 8-iso PGF₂α produced concentration-dependent contractions of rat aorta. When rings were pre-incubated with 0.3 uM 8-iso PGF₂α for 60 min, EDR were significantly impaired (Rmax control 76.1 ± 3.2% vs pre-incubated 46.2 ± 3.4%; $p<0.0001$). When 8-iso PGF₂α was washed out prior to measurement of EDR, Rmax did not differ from that seen in untreated aorta, but was significantly different from that in tissues pre-incubated without washout, $p<0.0001$ (Rmax untreated 63.6 ± 3.4% vs washed out 56.9 ± 3.6% vs non washed out 36.2 ± 2.9%). When diabetic tissue was pre-incubated with 8-iso PGF₂α, Rmax was not significantly different from that seen when non-diabetic tissue was similarly treated. **Conclusions:** 8-iso PGF₂α, at a concentration adjusted to reflect that found in diabetic plasma, caused significant endothelial dysfunction in non-diabetic rat aorta. This dysfunction was similar in extent to that seen in diabetic tissue and 8-iso PGF₂α did not cause further dysfunction in diabetic tissue. It is possible therefore, that increased circulating levels of 8-iso PGF₂α in diabetic vasculature, contribute to the endothelial dysfunction seen in diabetes.

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Distinct impact of insulin on vascular reactivity to angiotensin II and norepinephrine stimulation.

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Background and Aims: The action of insulin's vasodilation was observed by numerous studies although hyperinsulinaemia caused the sympathetic activation and facilitated angiotensin II (Ang II) action. We proposed that paradox effect of insulin on vascular reactivity may be related to different vasoconstrictors and endothelium status.

Materials and Methods: The thoracic aorta was prepared from male Wistar rat. Vascular reactivity on aortic rings was measured using an isometric force transducer. The aortic rings were equilibrated in Krebs-Henseleit buffer for 60 minutes and resting tension was set to 2 gramm. In some experiments, the endothelium was mechanically removed.

Results: A dose response curve to insulin (10, 30, 50 µU/ml) was tested in the presence of Ang II (10-6 mol/l) and norepinephrine (NE, 10-7 mol/l). In the presence and absence of endothelium, insulin inhibited Ang II induced vasoconstriction in dose-dependent manner. Vascular reactivity to Ang II stimulation was significantly diminished in the aortic ring with intact endothelium (Insulin 50 µU/ml: 0.19±0.04 vs 0.48±0.05 gramm, $P<0.01$). Contrary to Ang II, a dose-dependent inhibitory effect of insulin on vascular reactivity to NE stimulation was not observed in the either presence or absence of endothelium. NE response was not changed by intact or denuded endothelium (Insulin 50 µU/ml: 0.80±0.08 vs 0.98±0.06 gramm, $P>0.05$).

Conclusions: It concluded that the effect of insulin on vascular reactivity was depended on agonists used. Endothelium status had a marked impact on Ang II, whereas no effect could be observed using NE in the presence of high concentration of insulin (Supported by NSFC 39725013).

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Effects of Acute Hyperglycemia on Platelet Activation In Type 2 Diabetes.

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To establish the risk of acute, transient hyperglycemia (H) on thrombotic events in vivo in Type 2 DM (T2DM), 12 T2DM patients on diet and/or oral drugs (age 60±2 years, DM duration 7±1.2 years, BMI 29±0.5 kg/h², HbA1c 7.12±0.2%) were studied on 2 occasions at euglycemia (E) (~100 mg/dl) or H (plasma glucose, PG, ~250 mg/dl) for 4 h (randomized, cross-over, double blind study, hyperinsulinemic-E or -H clamp technique). Shear stress-induced platelet activation, plasma von Willebrand factor (vWF) antigen and RiCof, urinary 11-dehydroTxB₂, bleeding time and appearance of activation antigens (P-Selectin) on the surface of platelets in the bleeding time blood, were measured before, and at end of E and H studies. Plasma insulin was superimposable in E and H studies (~180 pmol/l). Expression of P-selectin increased after H (post vs pre: 1st min=+42.7±14.7%, 2nd min=+87.1±22.2%, 3rd min=137.4±45.6%, $p<0.05$), not after E ($p=NS$). Shear-stress induced greater platelet activation after H (closure time = 49.2±1.9 sec vs 41.5±1.7, $p=0.0012$; platelet retained 20-40 sec = 81.9±3.5% vs 87.1±3.2%, $p=0.0002$), not E ($p=NS$). Plasma vWF:Ag increased after H (pre 100.4±10.5%, post 144.3±13%, $p<0.0023$) as well as vWF:RiCof (pre 81.9±6.7, post 124.5±17.33, $p=0.043$), but not after E. Urinary 11-dehydroTxB₂ increased uring H, not E ($p=0.02$). **Conclusions:** in T2DM in reasonably good glycemic control, short-term hyperglycemia induces activation of platelets exposed to high shear stress in vitro (filtration method) or in vivo (bleeding time). Platelet activation is also reflected by increased urinary excretion of 11-dehydroTxB₂, likely related to increased vWF. Thus, acute hyperglycemia and moderate hyperinsulinemia, closely mimicking the post-prandial situation, may increase the risk for vascular occlusions by facilitating platelet activation, even in well controlled T2DM patients.

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DIABETIC PLATELETS ARE LESS SENSITIVE TO ACETYSALICYLIC ACID (ASPIRIN®) - THE POSSIBLE ROLE OF GLYCAEMIC CONTROL

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Aspirin (acetylsalicylic acid, ASA) is the most commonly used antiplatelet drug. It is most often recommended in the prevention of platelet-derived thrombosis in cardiovascular disease, also in diabetic patients. ASA is known to attenuate blood platelet reactivity by the irreversible inhibition of platelet cyclooxygenase. We are much less aware of the ability of aspirin to non-selectively acetylate all susceptible amino and hydroxyl groups in a plethora of other platelet and plasma proteins. Thus, a nonspecific acetylation competes with other nonenzymatic modifications of proteins in diabetes mellitus, like nonenzymatic glycosylation: amino residues previously occupied by glucose are available for acetylation. The susceptibility and/or vulnerability of various surface membrane receptors on blood platelets to acetylation might underlie the apparently differentiated sensitivity of blood platelets to ASA and the so-called "aspirin resistance". **Objective:** Our aim was to estimate the extent of decreased sensitivity of diabetic platelets to ASA and to monitor the relationship between the extent of protein glycation and the reduced platelet sensitivity to aspirin in patients with diabetes. **Methods:** We measured platelet ability to adhere and aggregate in the presence of agonists acid and used platelet function analyzer (PFA-100™, Dade Behring), whole blood impedance aggregometry and plasma turbidimetric aggregometry to monitor platelet response induced by collagen, arachidonic acid and ADP in 20 normal healthy volunteers (46±12 yr) and 27 insulin-treated patients with type 2 diabetes mellitus (49±8 yr; HbA_{1c} 9.8±2.9%). **Results:** In majority of control subjects (74%) and minority (27%, $p<0.015$) of diabetic patients the intake of 150 mg ASA a day for 1 week reduced platelet adhesiveness and reactivity, as reflected by the prolonged PFA-100™ collagen/epinephrine closure time (CEPI-CT) (up to 165±12 s in diabetic vs. 259±11 s in control individuals, $p<0.05$). Also platelet aggregation in whole blood and plasma became much less reduced in diabetic patients in response to ASA: IC₅₀ was significantly higher in diabetic compared to control subjects (4.8±2.6 µg/ml vs. 2.3±1.9 µg/ml, $p<0.05$). The reduced response of diabetic platelets to ASA correlated with HbA_{1c} ($p<0.05$ or lower). **Conclusions:** We conclude that one of the factors underlying the reduced response of diabetic platelets to the clinical doses of ASA might be the extent of protein glycation in diabetes mellitus. In diabetic patients suffering from chronic hyperglycaemia the occupancy of amino groups by glucose moieties might contribute to the moderate or stronger resistance of diabetic platelets to aspirin. Thus, higher doses of aspirin need to be considered in antiplatelet therapy and/or cardiovascular prevention in diabetes mellitus. Moreover, the differentiated response of individual diabetic patients to aspirin validates the necessity of the individual evaluation of efficient aspirin doses in clinical practice.

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Regulation of PKCzeta and thromboxane A2 release in thrombin stimulated platelets

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Background and Aims: Protein kinase C (PKC)zeta binds to glut-4 and is involved in the glucose transport after stimulation with insulin. Since recent mice knockout experiments revealed that some aspects of insulin resistance are dependent on glucose transport PKCzeta may play an important role in the development of the metabolic dysregulation in type 2 diabetes mellitus. We have investigated the contribution of PKCzeta to the increased pro-aggregatory properties of platelets of patients with type 2 diabetes mellitus compared to control persons. Thromboxane was measured as a potent hormone of platelet aggregation.

Materials and Methods: Platelets of patients with type 2 diabetes and control persons (n=6 respectively) were isolated by differential centrifugation. PKC extracts were separated into a cytosolic and a membrane fraction by a centrifugation at 100000 x g for 30 min. PKCzeta immuno blots were performed after treatment of equal numbers of platelets of patients and control persons with 1 U thrombin and without thrombin. Thromboxane B2 (TXB2) as the stable analogue of thromboxane A2 which is released by platelets was measured by ELISA (R & D, Abingdon). Regulation of PKCzeta was inhibited by the cell-membrane permeable pseudosubstrate peptide of the PKCzeta regulatory domain fused to myristic acid (pPKCzeta-myr). Statistical analyses were performed by Mann-Whitney U test.

Results: Thrombin stimulation of platelets for 5 min increased the TXB2 concentration from 105 +/- 15 pg/µl to 220 +/- 40 pg/µl (n = 6; $p<0.05$). This increase was completely abolished by co-incubation with pPKCzeta-myr. Incubation with pPKCzeta-myr alone had no effect on the basal thrombin release. Thrombin stimulation decreased the PKCzeta signal in immuno blots to nearly undetectable levels in the cytosol and membrane fraction (n=4). This regulation was seen in patients with type 2 diabetes and controls. Co-incubation of thrombin with pPKC-myr was able to antagonize the downregulation of the PKCzeta signal.

Conclusions: PKCzeta increased the formation of thromboxane A2 after stimulation of platelets. The regulation of the PKCzeta protein was unusual since its downregulation was the result of activation. PKCzeta is necessary for thromboxane release and but no differences between control persons and patients with type 2 diabetes were found.